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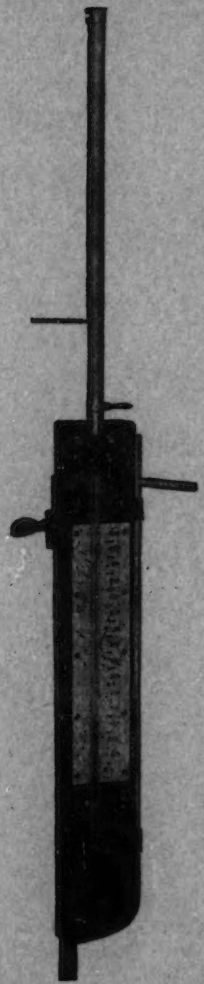

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VOLUNTARY ACCELERATION OF THE HEART

N. B. TAYLOR
IN COLLABORATION WITH H. G. CAMERON
From the Physiological Laboratory of the University of Toronto

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Two types of voluntary control of the heart beat are recognized, the indirect and the direct. By indirect control is meant the alteration in the rate of the heart which may be brought about by a subject who, being of a highly imaginative and susceptible nature, is able to conjure up scenes, ideas or experiences which under ordinary circumstances influence the heart rate. Such an individual voluntarily arouses emotional sensations and through these sensations influences his heart's action. Several cases of this nature have been reported in the literature, in some the rate was increased, in others it was slowed, by a self-induced emotional state. There is really nothing very extraordinary, from a physiological point of view, in such cases of indirect cardiac control. Of more interest to the physiologist and the clinician are those cases, mentioned by Carpenter, Frank and others, in which the mere direction of the subject's attention either by himself or by another to the heart causes a change in its rate. In some instances the rate is slowed by attention to the heart, in others it is accelerated. In the latter instances the cardiac acceleration is frequently evident to the patient himself and constitutes one form of palpitation.

The type of voluntary cardiac control which I am to report upon is that in which the subject accelerates his heart without any preliminary emotional effort, and in which mere direction of the attention to the heart is without effect upon its rate. Furthermore, the heart's accel-

eration is accompanied in many cases by dilatation of the pupils, constriction of the peripheral vessels or other phenomena associated with sympathetic excitation.

If one may judge from the few cases reported in the literature, direct voluntary control of the heart is a rare accomplishment. The first case, so far as we are aware, was reported by Tuke (1) in 1872 in his essay entitled, *The influence of the mind upon the body in health and disease*. The subject, aged 72, and a Fellow of the Royal Society, was able by an effort of the will to accelerate his heart from 63 to 82, an increase of 19 beats per minute. No records are given of hand volume or of blood pressure and no mention is made of any sympathetic phenomena. The subject when asked to describe the manner in which the acceleration was brought about, could scarcely do so but said that it seemed to be due to a "sort of impulse accompanied by an internal shiver." Tarchanoff (2) in 1885 reported a case of a medical student who could increase the heart rate from 72 to 120 per minute. Careful records were made in this case of blood pressure and hand volume. The pressure rose during the period of acceleration and the hand volume diminished. Tarchanoff states that the acceleration was entirely independent of any extraneous ideas and ascribes it to a special effort of the will. He states that he had met four other individuals who were able to accelerate the heart at will and in all these, as in the case he reports specifically, there was also control over the platysma, the muscles of the ear and the terminal joints of the fingers. He believes that alteration of vagal tone is not concerned in the production of the acceleration. He omitted, however, the important test of administering atropine before an attempted acceleration. Dilatation of the pupil is not mentioned. Pease (3) in 1889, reported the case of a student at the Harvard Medical School. This observer recorded the pulse acceleration by means of the sphygmograph. The beat was increased from 10.4 beats in 8 seconds during rest, to 12.7 beats per 8 seconds during acceleration. This subject could accelerate for short periods only—a few seconds at the most. He was able to contract his platysma myoides and ear muscles. The sweat glands were stimulated during the acceleration, the perspiration standing out in great drops upon the palms of the hands. No mention is made of vasoconstriction or dilatation of the pupil nor was the blood pressure recorded. Favill and White (4) recorded a case of voluntary heart acceleration, in 1917, associated with pupillary dilatation, constriction of vessels, sweating and muscular twitchings. This case is important in that the effect of atropine

administration upon the ability to accelerate the heart was investigated for the first time. This drug was found to be unable to remove the voluntary power which the subject exercised over the heart. Electrocardiographic tracings showed changes in regard to the height of the R. S. and T. waves and the length of the T–P interval which they point out are similar to the alteration in the form of the electrocardiogram which follows experimental excitation of the accelerator mechanism. The length of the P–R interval was unaltered. They believe that sympathetic stimulation is the responsible factor. Other cases of voluntary heart control have been reported by Van der Velde (5) and by Koeler (6), in neither of these was the atropine test performed. The most recent case, so far as we have knowledge of, was reported by West and Savage (7) in 1918. These authors recorded an acceleration of the heart from 69 to 96, an increase of 27 beats per minute. Atropine (0.002 gm.) administration reduced but did not destroy the ability to accelerate. They believe that reduction of vagal tone is the dominating influence in the acceleration. No mention is made of dilatation of the pupils or of peripheral vasoconstriction. The blood pressure was elevated during the voluntary effort.

History of the case. One of us (H. G. C.) is the subject of the investigations reported in this paper. He is a fourth-year student in Medicine at this University. He is 26 years of age, of excellent physique, and apparently in perfect health. In temperament he impresses one with being stolid and of tending toward the phlegmatic rather than the neurotic type. He served two years in the trenches, experiencing some of the most severe fighting. He is able by a direct volitional effort to accelerate the heart by from 40 to 55 beats per minute.

In 1917, while serving in the trenches in France, he was accustomed "to amuse the boys" by going through the pantomine exhibited by one of his fellows who had been returned to the base suffering from severe shell shock. He found that by visualizing the bursting of the shells and recalling their detonations he was able to arouse within himself the emotions of excitement and fright, and to enhance thereby the realism of his acting. He states that this mimicry left him very tired afterwards. No account was taken at this time of the pulse rate. In 1920 while studying physiology here he questioned the assertion that diminution of vagal tone was the dominating factor in the acceleration of the heart during muscular exercise and in excitement. By a play of the imagination he found that he was able to induce a state of excitement which closely simulated the spontaneous emotion. Upon

counting the pulse at these times he found it to be increased by several beats per minute. He next accustomed himself to fix his eyes upon a certain mark upon the wall while he was arousing the excited state, and found that after a time the mere direction of his attention to the mark together with an effort of the will, the precise nature of which he can scarcely describe, was sufficient to produce an acceleration of the heart. Later, by practice he was able to dispense with the visual association and to arouse the cardiac acceleration by an effort of the will alone.

SENSATIONS DURING THE ACCELERATION PERIOD. The subject states that he feels elated and experiences a sensation of tingling and warmth all over the body. He compares the sensation to that felt after partaking of a glass of wine. On one occasion at a dance he felt very tired and somewhat "seedy" in the early part of the evening, but upon accelerating the heart for very short periods several minutes apart, he after a time experienced a decided sense of well-being, his face became flushed, his eyes sparkled and he became talkative to a degree far beyond his usual nature. Toward the end of the evening his manner bordered on hilarity. These effects were so noticeable that his friends thought he had been "drinking," though he is practically a total abstainer. Upon cessation of the acceleration the subject experiences a sensation of intense relief. Prolonged periods of acceleration fatigued him both mentally and physically and at the end of days during which experiments were carried out, and consequently the periods had been long and frequently repeated, he became greatly exhausted and at night slept from 4 to 5 hours longer than was his custom. The acceleration, if much indulged in during the day, was followed by severe palpitation especially upon lying down, this in some instances was so intense as to prevent sleep, and was associated with air-hunger. Persistent occipital headache lasting sometimes for 12 hours or more was another unpleasant after-effect of the "stimulation."

CIRCULATORY EFFECTS: *Acceleration of the heart.* The subject is able at the word of command to increase immediately the rate of the heart from 50 to 70 per cent. The reaction time, that is, the time which elapses from the moment the word to accelerate is given by the observer to the first indication of increased rate, is very brief, being usually about the length of one normal beat. The rate does not attain its maximum speed at once but takes several seconds to do so (cf. table 1 and fig. 1). Upon the signal to stop the rate drops instantly to normal and remains so for from 6 to 10 seconds. The rate subsequently

TABLE 1

Experiment 3. Voluntary heart acceleration

PERIODS OF 6 SECONDS EACH		NUMBER OF HEART BEATS	RATE PER MINUTE
Normal rate before acceleration	1	7½	75
	2	7½	75
	3	7½	75
Heart acceleration	4	10	100
	5	10	100
	6	10	100
	7	11	110
	8	11½	115
	9	12	120
Acceleration stopped	10	7½	75
	11	7½	75
	12	7	70
	13	6½	65

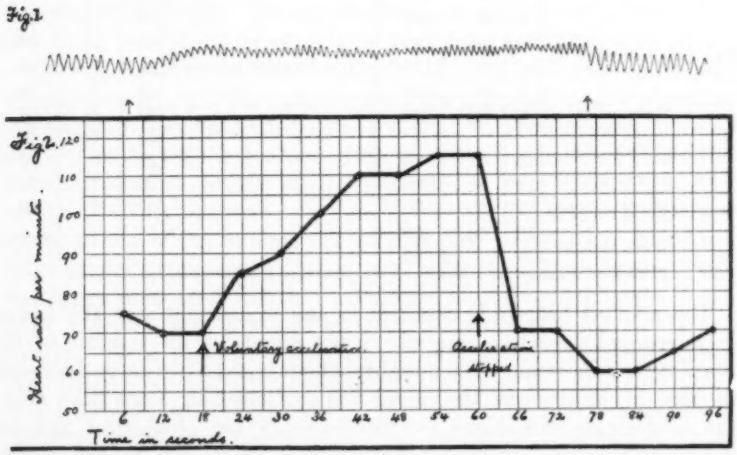

Fig. 1. Experiment 3. Voluntary heart acceleration. The arrows indicate the moments when the signals were given to accelerate and to cease accelerating, respectively.

Fig. 2. Graph showing the gradual increase in heart rate over an acceleration period of 48 seconds and the slowing of the rate below normal 6 to 20 seconds after acceleration.

drops below the normal and remains at 60 or 65 per minute for 25 seconds or so and then gradually increases until the normal is regained. In figure 2, the alterations in rate are shown in the form of a graph, the beats per minute being plotted along the ordinates and the time in 6-second periods along the abscissæ.

The amplitude of the pulse wave is diminished during the acceleration period but rises to normal again after the "stimulation" has ceased.

TABLE 2

Experiment 5. Voluntary heart acceleration

Record of the heart rate over a period of 2 minutes, 42 seconds showing the slowing of the rate below normal as an after effect.

PERIODS 6 SECONDS EACH		NUMBER OF HEART BEATS	RATE PER MINUTE	PERIODS 6 SECONDS EACH		NUMBER OF HEART BEATS	RATE PER MINUTE
Normal rate before acceleration.....	1	7½	75				
	2	7½	75		15	6	60
	3	7½	75		16	6	60
					17	6	60
Heart accelerated...	4	10	100		18	6½	65
	5	10	100		19	6½	65
	6	11	110		20	6½	65
	7	11	110	Acceleration stopped	21	7	70
	8	12	120		22	7	70
	9	12½	125		23	6½	65
					24	6½	65
	10	7	70		25	7	70
	11	7	70		26	7	70
Acceleration stopped	12	7	70		27	7	70
	13	6½	65				
	14	6	60				

The greatest heart-rate attained in any experiment was 12½ beats in a 6-second period—a rate of 125 per minute or an increase of 55 beats over the normal. The rate throughout is given in 6-second periods in table 2. In this case the record was continued for some time after the acceleration had ceased in order to study the after-effect and to determine the time required for the rate, after its fall, to regain the normal. Unlike the previous cases reported, the subject is able to maintain the accelerated rate for a considerable time. The usual duration of the acceleration in previous cases has been a few seconds or a minute at the most. A paroxysm of 3 minutes was common in our experiments and on one occasion the increased rate was continued for

a period of 7 minutes. On this occasion extreme fatigue was experienced and the trial was not repeated.

Slowing of the heart. Attempts at slowing of the heart were unsuccessful in our case. The subject believed that he could reduce at will the number of beats per minute but upon his being put to the test no evidence of slowing could be detected. We know of no undoubted instance in the literature in which slowing of the heart could be accomplished by a direct effort of the will. A moderate degree of slowing as we have already pointed out occurred as an after-effect of the acceleration. A very pronounced braoicardia in many instances occurred several hours after the accelerating efforts, especially if these had been frequently repeated during the day. On one occasion the rate dropped to 46 in the evening in another instance to 40. The reduced rate persisted for several hours but had disappeared by the following morning.

Other phenomena accompanying the cardiac acceleration. Dilatation of the pupil occurred invariably and was quite pronounced during the acceleration. There was frequently a rhythmical variation in the diameter of the pupil. During the effort, the muscles of the face, neck and limbs were the seat of irregular twitchings. These were particularly noticeable in the earlier experiments and have been mentioned by other observers. The question at once suggested itself "Are these the cause of the accelerated heart's action?" They seemed to us to be quite inadequate to produce such a marked acceleration but in order to rule them out, certain control experiments were undertaken. By means of a blood-pressure bag, encircling the limb and attached by tubing to one of the tambours of a Mackenzie polygraph, a record was taken of the twitchings simultaneously with the sphygmogram. The twitchings were then imitated as closely as possible and their record taken together with a record of the pulse without voluntary acceleration. There was no increase in pulse rate. Finally, the twitchings were able to be controlled almost entirely while the voluntary effort at acceleration was being made. The acceleration was unimpaired. The respirations were increased slightly during the acceleration. It is well known that increase in the respiratory rhythm causes an acceleration of the heart. That this is not a factor in the production of the tachycardia in the present instance was shown by forced breathing which increased the heart rate by 10 to 15 beats only and, on the other hand, by the subject's ability to prevent almost entirely the respiratory increase during the cardiac acceleration. It has previously been shown

by Tarchanoff, Pease and others, that the associated increase of the respirations is concerned to a slight extent only in the production of the acceleration in these cases. Sweating,which has been mentioned by several observers to be an accompaniment of the acceleration, was absent in this case.

The effect of atropine upon voluntary acceleration. A record was taken of the normal pulse rate and of the rate during acceleration; in the former case it was 75 and in the latter 125 beats per minute. Atropine, $\frac{1}{30}$ grain, was then given hypodermically. Within 12 minutes slight dryness of the mouth was noticed. No change in pulse rate occurred until 20 minutes had elapsed when it increased to 80 beats per minute. The maximum effect of the atropine was developed in about one hour. The pulse at this time was 90, the pupils were slightly dilated but reacted readily to light and there was no paralysis of accommodation. The nose, mouth and throat were intensely dry, the patient being scarcely able to speak. Pressure upon the vagus in the neck was now without effect upon the heart rate although such an effect could be observed normally in this subject. The heart at this time was voluntarily accelerated. The rate rose to 115 beats per minute, an increase of 25 over the rate already existing under the influence of atropine. This was the highest rate possible in the three attempts at acceleration which were made in the experiment, and as may be noticed failed by 10 beats to attain the level which was possible before atropine had been administered. Furthermore, under the influence of atropine the ratio existing between the rate before acceleration was commenced and the rate during the acceleration period is much less than in cases where the drug was not employed. Atropine, then, according to our experience, does disturb the voluntary control over the heart rate. Strenuous exercise while the subject was under the influence of atropine caused the rate to increase to 118 beats per minute (cf. table 3).

Blood pressure changes during the acceleration. Systolic and diastolic pressures were taken by Korotkoff's method. Three readings were first taken at 2-minute intervals before the acceleration. The heart was then voluntarily accelerated for 1 minute and readings taken thereafter each minute for a period of 4 minutes. The data for this experiment are tabulated below (table 4). It will be seen from the table that the pulse pressure is increased from 42 before to 60 during the acceleration. Since acceleration of the heart without an increase in the amount of blood ejected per beat would tend to lower the pulse pressure and the vasoconstriction, which we shall see also occurs

during the voluntary effort, would have a similar effect, the fact of the pulse pressure rising during the paroxysm suggests that the heart's action is augmented as well as accelerated. In all our observations upon the blood pressure, the pulse pressure was increased in this manner. Though no mention is made of this point in previous work we find upon examination of the data in the paper of West and Savage that the pulse pressure in these cases also increased by 20 per cent

TABLE 3

Experiment 7. Effect of atropine administration upon voluntary acceleration

TIME	PULSE	REMARKS
a. m.		
11:15	70	
11:16	125	Voluntary acceleration
11:17	70	
11:25	70	Atropine 1/30 grain
11:36	70	Slight dryness of mouth
11:46	80	
11:56	88	Throat, nose and mouth very dry
p. m.		
12:06	86	Pupils slightly dilated
12:11	88	
12:21	90	Mouth and throat so dry speaking is difficult. Pupils distinctly dilated but react readily to light and accommodation
12:25	115	Voluntary acceleration
12:30	118	Exercise—running upstairs
12:36	90	
12:40	88	
12:41	116	Voluntary acceleration
12:45	84	
12:50	115	Voluntary acceleration
12:55	86	Mouth a little less dry

during the acceleration. In Favill and White's case it remained practically the same or fell slightly.

Blood flow measurements. Estimations were made by the calorimetric method of Stewart upon the blood flow through the hands before, during and after the acceleration period. In all cases there was a sharp curtailment in the flow during acceleration. The observation was carried out in the following manner: the blood flow was measured for a period of 10 minutes prior to the commencement of the acceleration so that the normal flow might be determined to serve as a

base-line. The heart was then voluntarily accelerated for one 5-minute period and one 4-minute period, a rest of 1 minute being allowed

TABLE 4

Experiment 7. Effect of voluntary acceleration on the blood pressure
Subject, H. G. C.
June 2, 1921.

CONDITION	TIME	BLOOD PRESSURE IN MM. Hg.			PULSE
		Systolic	Diastolic	Pulse pressure	
	p. m.				
Normal before acceleration.....	3:36	118	76	42	70
	3:38	115	73	42	70
	3:40	118	76	42	70
Heart accelerated for 1 minute	3:41½	150	90	60	120
After acceleration.....	3:42½	125	80	45	70
	3:43½	120	80	40	68
	3:44½	119	76	43	70
	3:45½	120	76	44	70

TABLE 5

Experiments 10, 11, 12, 14, 15 and 16. The effect of the acceleration upon the blood flow

SUBJECT	DATE	GRAMS OF BLOOD PER 100 CC. OF TISSUE PER MINUTE						ROOM TEMPERATURE	MOUTH TEMPERATURE
		Right hand			Left hand				
		Before stimulation	During stimulation	After stimulation	Before stimulation	During stimulation	After stimulation		
		10 min.	*10 min.*	*10 min.*	*10 min.*	*10 min.*	*10 min.*	°C.	°C.
H. G. C.	7/6/21	10.1	4.4	4.3	8.9	5.5	5.4	18.3	37.11
	8/6/21	7.8	4.7	2.9	6.7	4.5	4.7	19.0	36.88
	10/6/21	12.8	11.1	11.1	11.8	9.3	10.3	21.0	37.11
	11/6/21	12.7	11.0	11.5	10.3	7.9	11.1	22.3	36.77
	13/6/21	15.6	13.1	10.0	12.6	10.3	10.3	23.5	37.25
	9/6/21	9.5	8.1	7.6	9.7	7.3	9.4	19.5	36.72
	14/6/21	12.0	13.3	12.1	12.8	11.9	10.3	22.0	37.05

between each paroxysm. These periods of acceleration and the intervening period of rest made up a total of 10 minutes. The estimation of the flow was continued for a further ten minutes in order to study the after-effect.

Upon the commencement of the acceleration there was an immediate sharp reduction in the blood-flow through the hands. The blood-flow curve continued to decline throughout the 10-minute period of acceleration but at the termination of this period the fall in the curve became arrested abruptly and continued either at the level of its arrest or rose again (see table 5). The curtailment of the flow during the period of acceleration in view of the changes in blood pressure indicates, we believe, a pronounced constriction of the vessels of the hand.

OBSERVATIONS UPON THE SUGAR OF THE BLOOD AND THE OCCURRENCE OF GLYCOSURIA DURING THE PERIOD OF ACCELERATION. Since a number of the effects, e.g., dilatation of the pupil and constriction of peripheral vessels, accompanying the heart acceleration were undoubtedly sympathetic phenomena, we decided to investigate whether any evidence of stimulation of the glycogenolytic function of the liver could be obtained by examination of the blood and urine after the paroxysm. The question was approached in the following manner. Preliminary estimations of the sugar in the blood and urine were carried out at 9, 11 and 1 p.m. for three successive days. During this time the subject partook of light teas containing like quantities of carbohydrate, proteins and fats on the evenings previous. He came to the laboratory breakfastless, and after the last estimation was made each day, ate a dinner containing approximately constant values of the different foodstuffs. On the fourth morning the heart was accelerated for from 2 to 3 minutes at 8:50, 10:50 a.m. and 12:50 p.m. respectively. Estimations of the blood sugar were made at 9 and 11 a.m. and 1 p.m. and urinalyses at 8 and 10 a.m., 12 noon and 2 and 4 p.m. The urine was first tested by Benedict's qualitative method; if this revealed the presence of sugar quantitative estimations were then made. The Lewis-Benedict method was employed in the estimation of blood sugar. The same routine was followed with regard to the diet as on the days when the heart was not accelerated. On the "normal" days the sugar of the blood varied from 0.07 per cent to 0.14 per cent and no sugar could be detected in the urine. On the day that the heart was accelerated the blood showed a sugar content of from 0.07 per cent to 0.08 per cent. Sugar appeared in the urine in the 12 noon and 2 p.m. analyses to the extent of 0.6 per cent and 0.5 per cent respectively in one experiment and 0.3 per cent and 0.2 per cent respectively in another (see table 6). The experiments seem to show quite definitely that the sugar of the blood is not increased during the "stimulation" nor does it seem to be reduced, and that sugar appears in the urine 1 to 3 hours

after "stimulation." The glycosuria lasts for from 2 to 3 hours, the urine being free from sugar 3 hours after the last effort at acceleration. The appearance of sugar in the urine is difficult to reconcile with a normal sugar level in the blood although lowering of the renal threshold from nervous influences suggests itself as a possible explanation. It may be that our estimations were not taken at proper intervals to reveal any resulting hyperglycemia. Unfortunately, circumstances so far have prevented our repeating these observations but this point will be kept in view for future investigations.

Discussion. The consensus of opinion favors the view that in these cases of voluntary acceleration the increase in rate is not the result, chiefly, at any rate, of a depression of vagal tone, but is due on

TABLE 6

Glycosuria following a series of paroxysms

DATE	CONDITION	BLOOD SUGAR			PER CENT SUGAR IN URINE				
		9 a.m.	11 a.m.	1 p.m.	8 a.m.	10a.m.	12 noon	2 p.m.	4 p.m.
1921			*per cent*	*per cent*					
June 17	Normal		0.100		Nil	Nil	Nil	Nil	Nil
June 18	Normal	0.120	0.140	0.120	Nil	Nil	Nil	Nil	Nil
June 20	Normal	0.080	0.066	0.066	Nil	Nil	Nil	Nil	Nil
June 21	Stimulation	↑0.080	↑0.075	↑0.067	Nil	Nil	0.6	0.5	Nil
June 23	Normal	0.069	0.075	0.068	Nil	Nil	Nil	Nil	Nil
June 24	Normal	0.088	0.088	0.088	Nil	Nil	Nil	Nil	Nil
June 25	Stimulation	↑0.089	↑0.088	↑0.076	Nil	Nil	0.3	0.2	Nil
June 29	Normal				Nil	Nil	Nil	Nil	Nil
June 30	Normal				Nil	Nil	Nil	Nil	Nil

the other hand to a direct action of the accelerator nerves. The inability of atropine to abolish the voluntary accelerations and the association of pupillary dilatation, sweating and vasoconstriction with the increased heart rate constitute the basis for this view. The evidence however is not entirely in accord with this conception. For instance we have found, as have West and Savage, that though atropine did not abolish the control entirely it seriously disturbed it and even in Favill and White's case, the increase in rate after atropine was only 28 beats per minute whereas the greatest increase which was possible without atropine was 63 beats per minute. If one had any assurance that atropine in the dosage given completely paralyzed the vagus

endings then any acceleration after its administration would undoubtedly be due to accelerator impulses. We have no such assurance. Pressure upon the vagus in the neck is at best a rough method of determining whether or not the drug has developed its full effect. Again, Gasser and Meek (8) have shown that removal of the stellate ganglia does not prevent the cardiac acceleration which accompanies strenuous exercise whereas vagal section does. They conclude that a reduction of vagal tone is the dominating factor in the production of the increased heart rate during exercise. Our results indicate that atropine reduces the voluntary acceleration in about the same degree as it does the acceleration due to exercise.

The associated sympathetic phenomena, however, must be considered in any judgment as to the mode of production of the acceleration; also the fact that the electrocardiogram taken during the voluntary acceleration is, as pointed out by Favill and White, similar in form to that shown by Rothberger and Winterberg (9) to be produced by electrical stimulation of the stellate ganglia, indicates a sympathetic influence. It is probable that both factors, namely, depression of vagal tone and excitation of accelerator fibers, working together in a reciprocal fashion are responsible for the voluntary acceleration.

SUMMARY

1. A case of voluntary heart acceleration is described and a brief account is given of the manner in which the accomplishment was acquired.

2. Dilatation of the pupils, vasoconstriction and elevation of the blood pressure are associated with the increased heart rate.

3. Atropine administration depresses but does not abolish the power of voluntary acceleration.

4. Decided glycosuria occurs as an after-effect of a series of accelerator efforts.

5. The conclusion is drawn that the acceleration is due in part to the withdrawal of vagal tone and in part to excitation of the accelerators, the two influences being called into reciprocal action by the voluntary effort.

The writer wishes to thank Prof. J. J. R. Macleod for his direction and guidance in this investigation.

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ON THE RELATION OF BLOOD-VOLUME TO TISSUE-NUTRITION

I. The Effects of Hemorrhage on the Circulatory and Respiratory Response to Changes in the Percentage of Oxygen and Carbon Dioxide in the Respired Air

ROBERT GESELL, EDWARD BLAIR AND ROBERT T. TROTTER

From the Department of Physiology, University of California, Berkeley

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The effect of hemorrhage on various circulatory phenomena, such as pulse rate, pulse pressure, mean blood pressure, and volume-flow of blood, has been a subject of considerable experimentation. The enormous peripheral dilatation which occurs in the production of hydremic plethora, which may be unaccompanied by a rise in mean blood pressure, and the marked constriction accompanying hemorrhage, which not infrequently is associated with a rise in mean blood pressure, are some of the findings of various workers (1), (2), (3), (4), (5) bearing on the present investigation. It was shown by one of us (5) in experiments on the dog that a progressive hemorrhage almost invariably led to a progressive reduction in the volume-flow of blood through the submaxillary gland, the extent of the reduction in the flow varying with the animal. The maximum effect noted was a decrease of 65 per cent resulting from a hemorrhage amounting to 0.9 per cent of the body weight. Replacement of the lost blood with gum-saline brought the volume-flow back to or above normal.

If these data on the submaxillary gland of the dog are indicative of general circulatory conditions, they show that changes in blood-volume elicit changes in cardiac output out of all proportion to the accompanying alterations in blood-volume. Thus it would follow that the injection of gum-saline not only increases the nutrient-flow[2] (volume-flow

[1] Reported before The American Physiological Society, December, 1920.

[2] By nutrient-flow we do not mean only the flow of constituents directly usable by the tissues as food, such as oxygen, dextrose, etc., but also constituents of the blood which may neutralize and carry away waste products. The red cells and alkaline reserve of the plasma, for example, are significant in this connection as carbon dioxide carriers.

of blood × concentration of blood constituents) through the submaxillary gland, as established by direct experimentation, but through other tissues as well. If a reduction in the volume-flow of blood exerts a detrimental effect upon tissue-nutrition through a reduced nutrient-flow, the infusion of an inert solution after partial depletion of the circulating blood theoretically should improve the nutrient condition of the tissues. Upon these premises some theories were based by one of us relative to the origin, prevention and treatment of traumatic shock (5).

It is the purpose of this series of investigations to determine by the employment of various methods the relation of blood-volume to the nutrition of tissues.

The series of experiments described in this paper involve the circulatory and respiratory response of the dog to a gradual reduction in the percentage of oxygen in the respired air when the carbon dioxide is absorbed and when it is allowed to accumulate. From the intensive study which this subject has recently received at the Mineola Station (6) and from the work of other laboratories, we are familiar with the compensations which occur with a decrease in oxygen of the respired air. The organism attempts to maintain an adequate supply of oxygen to the tissues by increased pulmonary ventilation and increased volume-flow of blood. We, therefore, reasoned that if a certain ventilation and a certain flow of blood are essential for a normal gaseous exchange when the hemoglobin value is normal, then a reduced flow of diluted blood entailed by hemorrhage would lower the resistance to and, therefore, change the response to a reduction in the oxygen of the respired air and likewise to a combined reduction of oxygen and accumulation of carbon dioxide. Having determined the effects of hemorrhage, the effects of replacing the blood with gum-saline could be studied.

METHOD. Unanesthetized dogs were connected by means of a soft rubber mask to a Henderson rebreathing apparatus arranged to record accurately the total ventilation and to absorb or not to absorb the carbon dioxide eliminated in the expired air as the oxygen in the tank was consumed. The dog's nose was bound closely to the valves to reduce the dead space to a minimum. The animal was held in a comfortable position by one of us throughout the experiment. The eyes were bandaged to exclude visual stimuli. The dog was allowed to breathe through the open valves for five minutes prior to the onset of the experiment to avoid the effects of initial excitement. One liter of room air was allowed per kilogram of body weight. With this allot-

ment of air the experiments lasted from twenty-five to thirty minutes, during which time respiration and time in seconds were continuously recorded on smoked paper. In addition the pulse rate was determined at intervals of one to two minutes and air samples for analysis were drawn at approximately seven-minute intervals. (Henderson's modified Haldane gas analyser was used.)

Pulse rate, respiratory rate, total ventilation in cubic centimeters per kilogram of body weight per minute, and oxygen ventilation in 0.1 cc. per kilogram of body weight per minute were plotted on the ordinates against the percentage of oxygen in the respired air on the abscissae. By plotting the time on the ordinates against the percentage of oxygen on the abscissae an approximately straight line (oxygen line) was obtained. The points on the smoked record at which the respiratory response and the heart rate were determined were set off on the oxygen line, giving the value of the percentage of oxygen on the abscissae. This permitted the plotting of the response at any number of points against the percentage of oxygen in the respired air.

Of some seventy experiments 19 graphs are shown in this paper, in all of which the continuous line represents pulse rate per minute, the large solid black circles respiratory rate per minute, the small solid black circles total ventilation in cubic centimeters per kilogram of body weight per minute, and the other small circles oxygen ventilation in tenths of a cubic centimeter per kilogram body weight per minute. The ordinates from left to right are: column A, time in minutes; column B, respiratory rate; and column C, pulse rate, total ventilation and oxygen ventilation.

Results. *Dog 1.* Male, mongrel bull dog about 4 years old, in excellent condition, weight 17.7 kgm. Our first effort was to study the response of a normal dog to a progressive reduction in the oxygen of the respired air to determine whether a uniformity of response or some purely objective end point, such as collapse in man, might not be obtained. Dog 1 gave no indication whatever of passing into a state of unconsciousness but rather became progressively more restless as the oxygen fell below 9 per cent. Failing in our original purpose to obtain a purely objective end point, we encouraged the animal to the best of our ability to continue the experiment to its maximum endurance. This point at which the dog insisted on being disconnected with the apparatus we called the "breaking point."

Of the sixteen experiments performed on this dog seven were done before hemorrhage and nine after. These experiments are partially

summarized in table 1. The complete data of twelve experiments are graphically shown in figures 1, 2 and 3.

In the experiments done before hemorrhage neither the general response nor the percentage of oxygen at the breaking point (see table 1)

TABLE 1

Dog 1

EXPERIMENT	DATE OF EXPERIMENT	HEMORRAGE IN PER CENT OF BODY WEIGHT	RED CELL COUNT	VALUES WHEN RESPIRED AIR CONTAINED APPROXIMATELY 20 PER CENT OF OXYGEN				VALUES WHEN RESPIRED AIR CONTAINED 8 PER CENT OF OXYGEN				PER CENT OF OXYGEN IN THE RESPIRED AIR AT THE END OF EXPERIMENT
				Respirations per minute in round figures	Total ventilation in cc. per kgm. of body weight per minute	Tidal air in cc.	Pulse rate	Respirations per minute in round figures	Total ventilation in cc. per kgm. of body weight per minute	Tidal air in cc.	Pulse rate	
1	9/3			34	840	437	116	63	1650	465	160	6.50
2	9/4			23	540	380	87	39	1350	612		6.10
3	9/10			33	660	354	123	41	1650	703	159	5.85
4	9/14			53	965	322	100					7.26
5	9/21			41	901	390	83					8.03
6	9/22			37	1004	480	82	31	1470	826	165	5.85
7	9/22		8,201,000	44	824	330	88	54	1965	644	168	5.55
8	9/23	2.82		20	406	354	154	35	930	463	151	4.64
9	9/24		6,520,000	19	459	435	116	40	1350	597	162	5.05
10	9/25	1.78	5,600,000	15	338	408	140	42	1380	574	174	4.89
11	9/25		4,260,000	15	326	410	120	37	1230	580	174	4.35
12	9/27	1.78		20	554	500	124	38	1785	831	180	4.57
13	9/28	1.70	3,650,000	23	544	412	104	43	1350	549	154	5.02
14	9/29	1.80	2,852,000	18	431	415	90	33	885	474	105	3.81
15	9/30		2,672,000	20	568	512	106	43	1350	541	121	4.30
16	10/7		3,320,000	14	369	440	100	36	1080	531	137	5.32
Average figures before hemorrhage				38	962	384	97	45	1617	626	164	5.97
Average figures after hemorrhage				18	444	431	117	39	1260	571	151	4.66

shows a desirable regularity. The gross irregularity in the breaking point noted particularly in experiments 4 and 5, however, seems explainable. It can probably be attributed to contaminated food. At

least no more irregularities of the magnitude in experiments 4 and 5 occurred after instituting a regular diet of bread, dog-biscuit and freshly cooked liver. The importance of a regular and wholesome diet is a finding agreeing with the observations at the Medical Research Laboratory at Mineola on the relation of nutrition to compensation for high altitude.

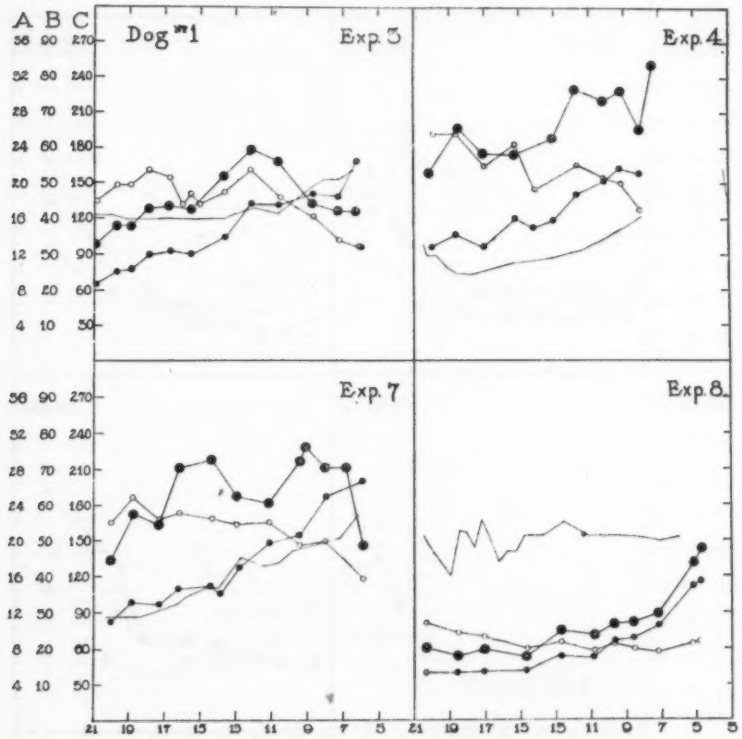

Fig. 1

Nine experiments were performed on dog 1 after hemorrhage, five immediately after hemorrhages amounting to 2.82, 1.78, 1.78, 1.70 and 1.80 per cent of the body weight. In the remaining experiments an interval of a day or more elapsed following hemorrhage. See table 1 and figs. 1, 2 and 3.) The blood was drawn by means of a large syringe needle inserted into the external jugular vein exposed under local anesthetic for a distance of one-half inch.

To our surprise the first hemorrhage amounting to 2.82 per cent of the body weight appeared to increase the tolerance of the animal to a lack of oxygen, in that it extended the breaking point from an average percentage of oxygen of 5.97 to 4.64. (See table 1 and fig. 1, exper. 8.) Unable to interpret these results, we were interested to determine the

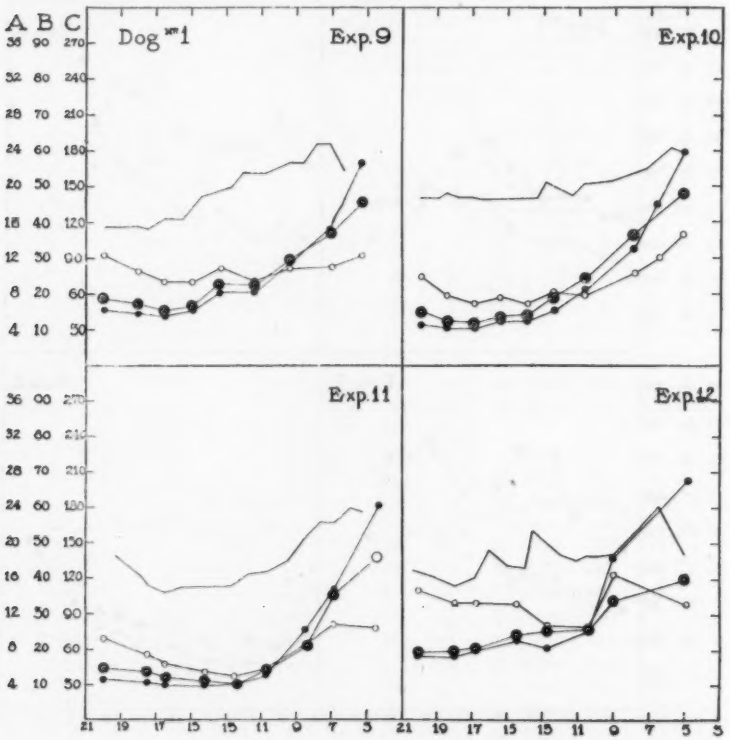

Fig. 2

effects of further massive hemorrhages. The animal was bled on four more occasions amounting to a total of 9.88 per cent of the body weight, the five hemorrhages reducing the red cell count from 8,201,000 to 2,672,000. The breaking point continued to occur at the same low percentage of oxygen, an average percentage of 4.66 after hemorrhage as compared with 5.97 before hemorrhage. Analysis of the graphs

and table 1 will show these and other points of difference; for example, the even response of the animal after hemorrhage as compared with the irregular response before hemorrhage. Further, if we compare the response of the animal at the beginning of each experiment when the respired air contains 21 per cent oxygen as summarized in table 1, we

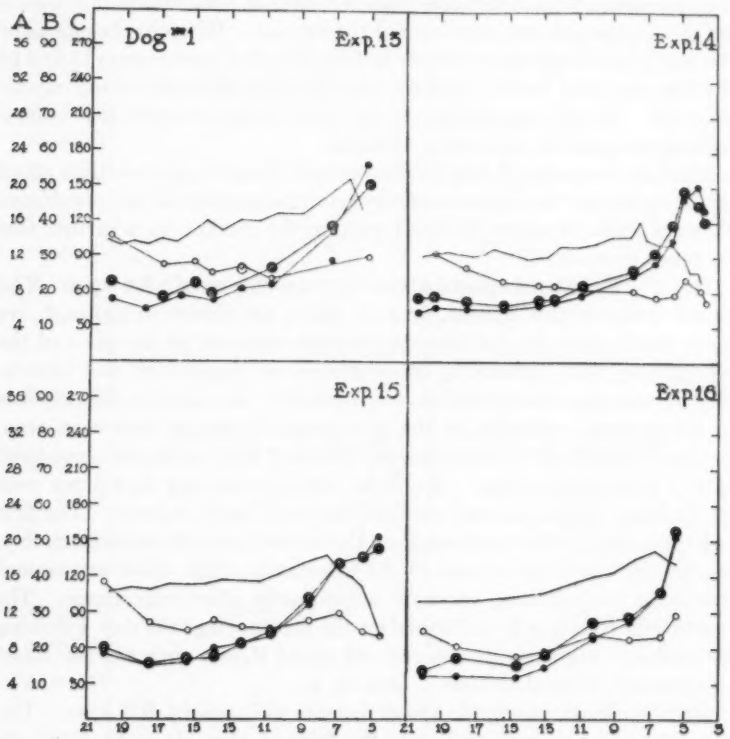

Fig. 3

find an average reduction in the respiratory rate from 38 respirations per minute before hemorrhage to 18.2 per minute after hemorrhage; a reduction in total ventilation from 962 cc. before hemorrhage to 444 cc. per kilogram of body weight per minute after hemorrhage; an increase of tidal air from 384 to 431 cc; and an increase of heart rate from 97 to 117 per minute. A comparison of the response of the dog near

the close of the experiments when respiring air containing only 6.5 per cent oxygen shows, with the exception of the pulse rate and tidal air a difference in figures in the same direction.

It should be mentioned that despite the fact that in the earlier experiments the graphs indicate no harmful effects of hemorrhage, we gained the impression from direct observation that each hemorrhage produced a decline in the general condition of the animal. Whereas the effects of the first hemorrhage were scarcely noticeable, after experiments 13 and 14 the dog was very weak. Indeed, we feared his death following experiment 14. In this experiment no breaking point occurred and unconsciousness came on without a struggle.

That the response of dog 1 indicates that hemorrhage has little effect on the resistance to a decrease of oxygen in the respired air is a conclusion we were loath to accept without putting the method to a further test on other animals.

Dog 2. Female, shepherd about 1½ years old, weight 8.6 kgm. The experiments on this animal, four of which are shown in figure 4, are more satisfactory in that unconsciousness occurred at the close of the rebreathing test, permitting observations on respiratory and circulatory decompensation as well as compensation. In addition the response to the gradual reduction in the percentage of oxygen was very even. In dog 2 we wished to determine the effects of what might be considered a very large hemorrhage. We drew blood amounting to 4.3 per cent of the body weight, almost one-half the total blood volume. The first two experiments were performed on the normal animal on the same day, one in the morning and one in the afternoon. The third experiment was done the following morning immediately after hemorrhage. The fourth experiment was performed on the afternoon of the day following hemorrhage, allowing, as the red cell count shows, time for the blood to approach normal volume. (See fig. 4.)

Dog 3. Male, mongrel, about 6 years old, weight 8.2 kgm. Unconsciousness occurred with only the faintest struggle so that the observations were prolonged here, too, into the stage of decompensation. The response under the three conditions described for dog 2 are shown in three graphs in figure 5; the first graph before hemorrhage, the second immediately after hemorrhage amounting to 3.11 per cent of the body weight, and the third the day following.

The similarity, almost superimposability, of the curves of the first two experiments on dog 2 attach significance to any changes which might occur as a result of hemorrhage, and we note in both dogs 2 and

3 that the initial pulse rate, respiratory rate, (not so striking in dog 2) total and oxygen ventilation are higher in the experiments performed immediately after hemorrhage than in those before. After time had elapsed for dilution of the blood the respiratory response in dog 3 resembled more nearly that prior to hemorrhage, the pulse rate, however,

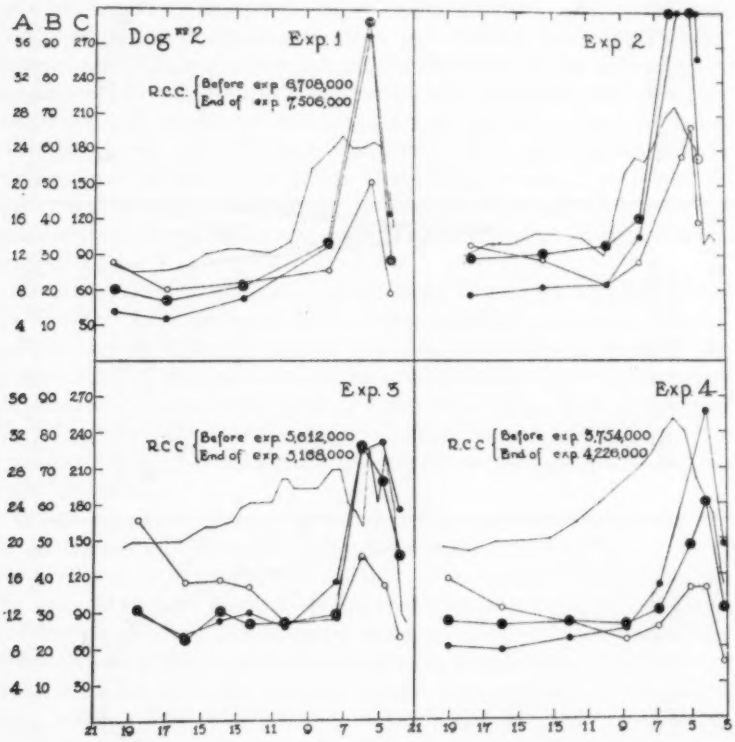

Fig. 4

remaining elevated. If we note the respiratory compensations beginning at a point where the respired air contained 9 per cent of oxygen and note also the broken compensation occurring where the air contains 5 per cent of oxygen, the differences in the nature of the response of dog 2 in the three conditions appear very slight indeed. In dog 3 the data are incomplete in the experiment before hemorrhage, in that the

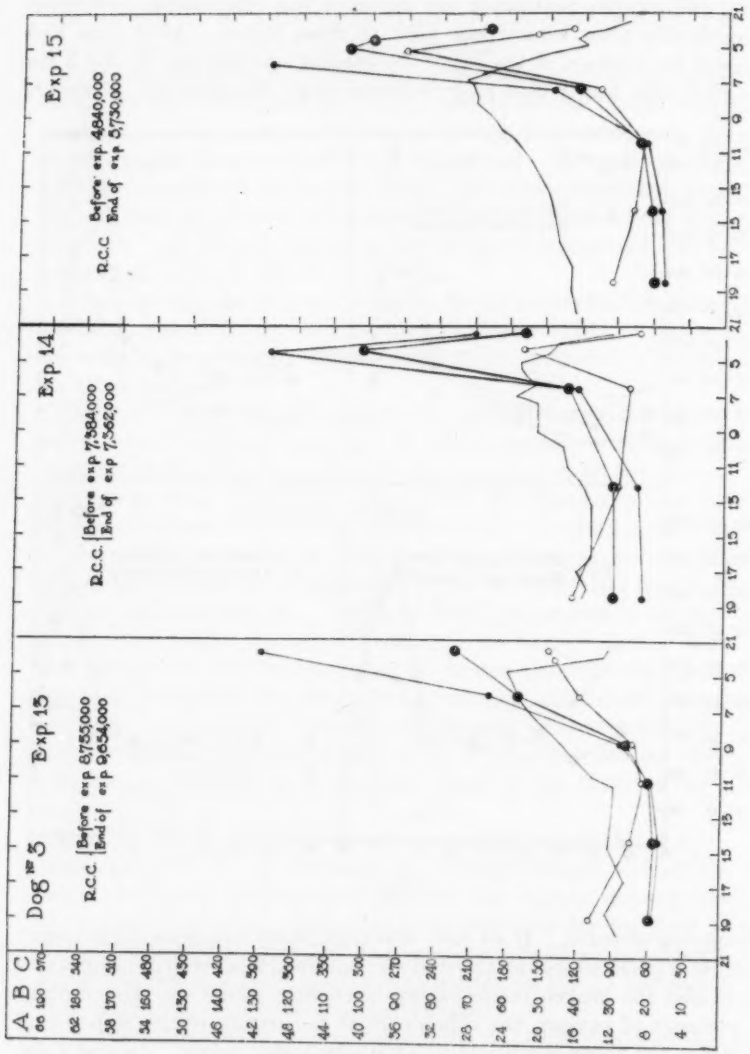
Dog № 5
Exp 13
R.C.C. Before exp 8,755,000
End of exp 9,654,000
Exp 14
R.C.C. Before exp 7,384,000
End of exp 7,562,000
Exp 15
R.C.C. Before exp 4,840,000
End of exp 5,730,000
A B C

Fig. 5

respiratory decompensation occurred abruptly at the end of the experiment, preventing a final observation for plotting. But barring this, it appears that the response of dog 3 is similar to that of dog 2 in the three conditions under study.

This similarity of the response obtained in dogs 2 and 3 under apparently similar conditions speaks well for the method. Yet comparison of the respiratory response of these two animals immediately after hemorrhage with the response of dog 1 also after hemorrhage shows a striking difference. Whereas in dogs 2 and 3 the ventilation was of higher frequency and volume at the beginning of the experiment following hemorrhage, in dog 1 the reverse occurred. Perhaps this difference in results can be sought in the nature of the dogs, for dog 1, compared to dogs 2 and 3, was an exceedingly nervous animal, apparently enjoying an excess ventilation which was reduced by the general quieting effect of hemorrhage.

To summarize the effects of hemorrhage, it may be said that while the elevated pulse rate noted at the beginning of all experiments following hemorrhage indicates a disturbance in circulation, analysis of the circulatory and respiratory response at the close of experiments when the organism is taxed to its utmost shows no striking difference in dogs 2 and 3 as a result of hemorrhage. In addition analysis of figure 1, experiment 8, dog 1 shows a postponement of the breaking point resulting from hemorrhage.

Whether such results can be taken to indicate that hemorrhage has no detrimental effects on the ability of the resting organism to withstand low pressures of oxygen in the respired air or whether we must doubt the reliability of our method, should be considered. It is possible that a lack of oxygen is not an adequate stimulus to demonstrate circulatory and respiratory disturbances resulting from hemorrhage, for the effect of hemorrhage upon the rate of oxygen consumption in the dog is considerable. The experiments on dogs 2 and 3 are given as examples. Inspection of the graphs from dog 2 shows the duration of experiments 1, 2 and 4 to be 26.8, 27.5 and 29.8 minutes, an average of 28 minutes as compared with 39.7 minutes duration immediately after hemorrhage. Since the same amount of air was allowed in each experiment and since approximately the same concentration of oxygen was reached at the end of each experiment, the oxygen consumption immediately following hemorrhage was 29 per cent below that occurring before and one day after hemorrhage. In dog 3 the duration of experiments 1 and 3 was 30 minutes each, compared with 36 minutes duration of the ex-

periment immediately after hemorrhage, indicating a reduction in oxygen consumption amounting to 17 per cent.

A fact possibly associated with the reduction in oxygen consumption is the effect of hemorrhage on the muscles. Hemorrhage invariably produced a palpable loss of muscular tone. Although the massive hemorrhages undoubtedly reduced the volume-flow of blood, and possibly to an extent which alone would account for the reduced oxidations, yet it must be admitted that a decrease in muscle tonus might in itself reduce the demands for oxygen, thereby reducing the consumption of oxygen. We know the effects of even the slightest amount of exercise on respiration at high altitudes, and if the reduced muscle

TABLE 2

RABBIT NO.	PER CENT OF OXYGEN IN THE RESPIRED AIR AT THE END OF THE EXPERIMENT BEFORE HEMORRHAGE	PER CENT OF CARBON DIOXIDE IN THE RESPIRED AIR AT THE END OF THE EXPERIMENT BEFORE HEMORRHAGE	RED CELL COUNT BEFORE HEMORRHAGE	HEMORRHAGE IN PER CENT OF BODY WEIGHT	PER CENT OF OXYGEN IN THE RESPIRED AIR AT THE END OF THE EXPERIMENT AFTER HEMORRHAGE	PER CENT OF CARBON DIOXIDE IN THE RESPIRED AIR AT THE END OF THE EXPERIMENT AFTER HEMORRHAGE	RED CELL COUNT AT END OF EXPERIMENT AFTER HEMORRHAGE
1	3.25	12.41	5,150,000	1.83	4.45	10.75	4,600,000
2	3.17	12.83	6,426,000	1.69	3.75	12.50	5,500,000
3	3.23	12.62	5,372,000	1.94	4.69	11.67	3,404,000
4	3.66	11.37	5,303,000	1.52	3.85	11.20	4,932,000
5	3.45	12.75	5,820,000	2.18	5.08	12.43	4,040,000

tonus is in part responsible for the decreased oxidations, it may also partially explain the respiratory response that occurs after hemorrhage. On the other hand, the failure of dogs to show greater response to low oxygen pressures after hemorrhage when we know their oxygen consumption is below normal may be due to the relative inadequacy of lack of oxygen as a respiratory and circulatory stimulus. Perhaps the response of an animal to a progressive accumulation of carbon dioxide in the respired air would be a more reliable index to the effects of hemorrhage.

We performed such experiments on the dog in which we allowed carbon dioxide to accumulate as the oxygen was consumed. Under these conditions the dogs became very restless so that a uniformity of response was unobtainable in the rebreathing tests, yet we gained the

impression that dogs after hemorrhage reacted more strongly to an accumulation of carbon dioxide in the respired air than did normal animals.

We tested these results further with rabbits which were put on a platform in a confined space under a bell jar inverted over water, allowing each rabbit about 1.85 liters of air per kilogram of body weight. The air in the bell jar was kept in circulation by means of a large rubber bulb connected with it. These animals rebreathed the air to a point of complete collapse without a struggle or sign of discomfort. When respiration became jerky and about to cease, a sample of gas was withdrawn for analysis and the animals removed. The rabbits were usually on their feet within 15 seconds after breathing room air. This type of experiment was performed before and after hemorrhage. Usually one day intervened between experiments on each rabbit. The data are shown in table 2. As indicated by this purely objective end point, hemorrhage, despite the rapid dilution of blood which is shown by the red cell count, reduced the tolerance to a combined reduction of oxygen and accumulation of carbon dioxide. We do not know the relative importance of these two factors of deficiency of oxygen and excess of carbon dioxide in rabbits. Attention should be called, however, to the fact that rabbits do have a high tolerance for carbon dioxide (7).

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ON THE RELATION OF BLOOD-VOLUME TO TISSUE-NUTRITION

II. The Effects of Graded Hemorrhage on the Volume-Flow of Blood Through the Striated Muscle of the Dog

ROBERT GESELL assisted by C. A. MOYLE

From the Department of Physiology of the University of California, Berkeley

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In the preceding paper (1) some effects of hemorrhage on the response of the unanesthetized dog and rabbit in rebreathing experiments were described. In these experiments we studied the effects of a reduction in the percentage of oxygen in the respired air and also the combined effects of a reduction of oxygen and accumulation of carbon dioxide. Apart from one experiment on the dog in which the total ventilation was reduced below normal by hemorrhage, hemorrhage had little effect upon the respiratory response to a reduction in the percentage of oxygen in the respired air. This absence of a change in the respiratory response occurred despite the fact that hemorrhage markedly reduced the consumption of oxygen by the animals. While the restlessness of dogs in rebreathing experiments in which carbon dioxide accumulated in the respired air as the oxygen decreased prevented a quantitative analysis of the effects of hemorrhage, it appeared that the animals were more restless in rebreathing experiments after hemorrhage than in experiments before hemorrhage. These results were reversed in experiments where carbon dioxide was absorbed, the animals being more restless in the experiments performed before hemorrhage than in those after hemorrhage. Rebreathing experiments on the rabbit in which the expired carbon dioxide accumulated and which were extended to the point of collapse showed detrimental effects of hemorrhage.

The probability was pointed out that the reduced consumption of oxygen after hemorrhage was due to a diminished flow of blood and that the absence of an increased respiratory response to a reduction in the percentage of oxygen in the respired air was due to the relative inadequacy of lack of oxygen as a respiratory stimulus.

[1] Preliminary report of this work will be found in the Proceedings of the Society for Experimental Biology and Medicine, 1921, vol. xix, p. 56.

In this connection it seemed desirable to determine to what extent hemorrhage affects the volume-flow of blood through striated muscle, for the work which is summarized above was based largely upon experiments performed on the submaxillary gland of the dog (2). In these experiments hemorrhage and injection produced marked changes in the volume-flow of blood, out of all proportion to the changes in blood-volume, but since the submaxillary gland comprises such a small amount of the total tissue of the organism it seemed necessary to secure quantitative data on the effects of changes in blood-volume on the flow of blood through a more representative tissue such as striated muscle. Bayliss (3) has already published some data on this subject, but a more detailed study seemed essential for the continuation of our problem.

METHOD. The volume-flow of blood through the leg muscles of the dog was measured. The usual procedure was to administer 0.4 cc. of a 2 per cent morphine sulphate solution hypodermically one-half to three-quarters of an hour before the experiment. In some cases these injections alone produced satisfactory anesthesia during the period of observation; in other cases a small amount of ether was administered through an ether bottle in the usual way. To record the blood-flow a cannula was inserted in the femoral vein, this being ligated a short distance below, allowing blood to enter it from any single lateral branch carrying a flow not too great for registration with the drop method. A modified drop method previously described (4) was used. Mean blood pressure and pulse rate were recorded with a mercury manometer connected with the left carotid artery. Blood was drawn either from this artery or the femoral artery at intervals of approximately ten minutes. The blood-volume was increased by the injection of a 6 per cent gum arabic suspension in a 0.9 per cent sodium chloride solution.

RESULTS. The effects of hemorrhages and subsequent injection on volume-flow of blood, mean blood pressure and pulse rate are shown in tables 1 to 5.

A comparison of the last four columns in experiments 1, 8, 9 and 10 shows the relative changes in blood-volume and volume-flow of blood resulting from hemorrhage and injection respectively. In experiments 2, 3, 4, 5, 6 and 7 the injection of gum-saline for some reason proved very toxic. (We shall publish a note on the toxicity of gum saline in one of our later papers.) The data, therefore, on the effects of increasing the blood-volume on volume-flow of blood are relatively incomplete. The relative changes in blood-volume and volume-flow of blood with decreasing blood-volume in these incomplete experiments are shown in columns 6 and 7.

In experiment 1 the injection of gum-saline was non-toxic and the regularity with which the effects of a progressive decrease in blood-volume was duplicated by subequent and equal increases in blood-volume is striking. The first two hemorrhages (1.02 per cent and 0.97 per cent of the body weight) lowered the volume-flow of blood from 15.7 to 10.4 and 3.0 drops per minute respectively. The two corresponding injections raised the volume-flow of blood from 3.0 to 10.0 and 15.6 drops per minute respectively. The first two hemorrhages raised the pulse rate from 112 to 182 and 224 per minute respectively and lowered the mean blood pressure from 100 to 92 and 72 mm. Hg. respectively.

TABLE 1

Experiment 1

1	2	3	4	5	6	7	8	9	10
OBSERVATION	HEMORRHAGE IN PER CENT OF BODY WEIGHT	INTRAVENOUS INJECTION IN PER CENT OF BODY WEIGHT	VOLUME-FLOW OF BLOOD IN DROPS PER MINUTE	MEAN BLOOD PRESSURE IN MM. HG.	PULSE RATE	PERCENTILE REDUCTION OF BLOOD VOLUME, ASSUMING THE BLOOD-VOLUME TO BE 9 PER CENT OF THE BODY WEIGHT	PERCENTILE REDUCTION OF VOLUME-FLOW OF BLOOD	PERCENTILE INCREASE IN BLOOD-VOLUME ABOVE THAT IN CIRCULATION AFTER LAST HEMORRHAGE	PERCENTILE INCREASE IN VOLUME-FLOW OF BLOOD
1			15.7	100	112				
2	1.02		10.4	92	182	11.4	33.7		
3	0.97		3.0	72	224	22.2	81.0		
4		1.00	10.0	88	152			14.3	33.3
5		1.00	15.6	90	100			28.6	52.0
6		0.75	17.0	96	100			39.3	56.6
7	0.80		12.3	90	135	8.8	6.2		
8	0.72		5.8	80	224	16.8	16.6		

The two corresponding injections lowered the pulse rate from 224 to 152 and 100 per minute respectively and raised the mean blood pressure from 72 to 88 and 90 mm. Hg. respectively. A comparison of columns 9 and 10 and 11 and 12 shows that hemorrhage and infusion changed the volume-flow of blood out of all proportion to the change in blood-volume.

Examination of the tables reveals that each hemorrhage in every experiment invariably reduced the volume-flow of blood. The effect, however, varied considerably with the animal. In the first six experiments it will be noted that the volume-flow of blood was decreased out of proportion to the decrease in blood-volume. This held for all

hemorrhages, the initial, as well as the final. In the next experiment (exper. 7) the reduction in blood-volume and volume-flow were equal in the first two hemorrhages, but in the last two the volume-flow was decreased out of all proportion to the decrease in blood-volume. The results in experiment 8 are comparable to those in experiment 7. The reduction in volume-flow was only slightly in excess of the reduction in blood-

TABLE 2

1	2	3	4	5	6	7
OBSERVATION	HEMORRHAGE IN PER CENT OF BODY WEIGHT	VOLUME-FLOW OF BLOOD IN DROPS PER MINUTE	MEAN BLOOD PRESSURE IN MM. HG.	PULSE RATE	PERCENTILE REDUCTION OF BLOOD-VOLUME, ASSUMING THE BLOOD-VOLUME TO BE 9 PER CENT OF THE BODY WEIGHT	PERCENTILE REDUCTION OF VOLUME-FLOW OF BLOOD
Experiment 2						
1		62.0	124	84		
2	1.03	46.0	124	180	11.4	25.8
3	0.62	30.0	116	312	18.7	51.6
4	0.62	20.4	88	352	27.3	83.2
Experiment 3						
1		20.3	98	132		
2	0.95	17.2	92	150	10.5	15.3
3	0.82	15.4	94	192	19.4	24.1
4	0.90	12.0	80	200	26.9	40.9
5	0.54	7.2	52	196	32.8	64.5
Experiment 4						
1		89.0	114	98		
2	0.76	65.5	91	108	8.4	26.4
3	0.61	61.0	72	133	15.2	31.5
4	0.58	38.5	53	106	21.6	56.8

volume in the first two hemorrhages, but again in the last two the reduction in the volume-flow greatly exceeded the reduction in blood-volume. In experiment 9 the first hemorrhage produced a negligible reduction in the volume-flow of blood, results comparable to those described by Eyster and Meek (5) who used the x-ray shadow of the heart to determine the effects of hemorrhage on cardiac output. Though exerting no effect upon the volume-flow, this initial hemorrhage

which was relatively small had a strikingly accelerating effect on the pulse rate, increasing it from 104 to 236 per minute. The following two hemorrhages elicited large reductions in the volume-flow disproportionate again to the reduction in the blood-volume. The second

TABLE 3

1	2	3	4	5	6	7
OBSERVATION	HEMORRHAGE IN PER CENT OF BODY WEIGHT	VOLUME-FLOW OF BLOOD IN DROPS PER MINUTE	MEAN BLOOD PRESSURE IN MM. HG.	PULSE RATE	PERCENTILE REDUCTION OF BLOOD-VOLUME, ASSUMING THE BLOOD-VOLUME TO BE 9 PER CENT OF THE BODY WEIGHT	PERCENTILE REDUCTION OF VOLUME-FLOW OF BLOOD
Experiment 5						
1		66.3	92	108		
2	1.00	43.0	73	114	11.0	35.0
3	0.49	34.3	55	148	16.5	48.0
4	0.51	25.4	39	146	22.2	61.2
Experiment 6						
1		60.0	124	68		
2	0.68	45.0	132	82	7.7	25.0
3	0.58	26.5	132	88	14.3	55.8
4	0.62	19.3	126	146	20.7	68.0
5	0.62	17.7	103	240	28.2	70.5
6	0.54	10.1	48		34.3	83.1
Experiment 7						
1		55.5	139	82		
2	0.98	50.0	137	98	10.0	10.0
3	0.64	45.5	129	125	18.0	18.0
4	0.44	33.0	110	292	23.0	40.5
5	0.42	12.7	89	580	27.7	77.0

hemorrhage amounting to only 0.53 per cent of the body weight elicited a decrease in volume-flow amounting to 45.6 per cent of the normal flow. In experiment 10 the reduction in volume-flow remained below or approximately equalled the reduction in blood-volume in the first four hemorrhages, the greater reduction occurring only after 35.2 per cent of the total blood had been drawn.

TABLE 4

1	2	3	4	5	6	7	8	9	10
OBSERVATION	HEMORRHAGE IN PER CENT OF BODY WEIGHT	INTRAVENOUS INJECTION IN PER CENT OF BODY WEIGHT	VOLUME-FLOW OF BLOOD IN DROPS PER MINUTE	MEAN BLOOD PRESSURE IN MM. HG.	PULSE RATE	PERCENTILE REDUCTION OF BLOOD-VOLUME, ASSUMING THE BLOOD-VOLUME TO BE 9 PER CENT OF THE BODY WEIGHT	PERCENTILE REDUCTION OF VOLUME-FLOW OF BLOOD	PERCENTILE INCREASE IN BLOOD-VOLUME ABOVE THAT IN CIRCULATION AFTER LAST HEMORRHAGE	PERCENTILE INCREASE IN VOLUME-FLOW OF BLOOD
Experiment 8									
1			37.0	116	66				
2	0.68		33.4	113	70	7.5	9.7		
3	0.69		30.5	110	132	15.2	17.6		
4	0.71		20.0	96	212	23.0	46.0		
5	0.44		8.4	64	176	28.0	75.0		
6		0.45	8.8	64	152			6.9	4.7
7		0.68	41.8	80	144			17.4	398.0
8		0.68	66.0	89	146			27.8	686.0
9		0.68	66.0	103	136			38.2	686.0
10		0.68	66.0	114	120			48.6	686.0
11	0.68		52.0	94	174	7.0	12.1		
12	0.72		26.0	62	176	14.5	60.3		
13	0.45		15.0	39	180	19.1	77.0		
Experiment 9									
1			129.0	118	104				
2	0.68		128.0	131	236	7.7	0.8		
3	0.53		69.0	100	300	13.6	46.5		
4	0.32		28.8	62	296	17.1	77.6		
5		0.32	39.4	61	260			4.2	36.8
6		0.53	112.0	70	236			11.4	288.8
Experiment 10									
1			37.0	83	103				
2	1.05		35.7	89	123	11.7	4.5		
3	0.54		32.0	86	138	18.5	13.5		
4	0.51		28.0	86	160	23.5	24.3		
5	0.48		27.0	80	196	28.5	27.0		
6	0.56		14.8	74	222	35.2	59.5		
7	0.51		5.8	60	246	40.8	83.8		
8		0.64	18.3	64	208			13.3	215.5
9		0.64	25.7	44				26.6	343.1

The later hemorrhages invariably had marked effects both on volume-flow of blood and mean blood pressure. The effects of the initial hemorrhages, as was apparent from the results described, were not so constant. The data on the effects of the initial hemorrhages of the ten experiments are summarized in table 5. Initial hemorrhages ranged from 0.68 per cent to 1.03 per cent of the body weight. Each initial hemorrhage elicited a reduction of the volume-flow of blood and an acceleration of the pulse. The extent of these changes varied with

TABLE 5

1	2	3	4	5	6	7	8
EXPERIMENT	FIRST HEMORRHAGE IN PER CENT OF BODY WEIGHT	VOLUME-FLOW OF BLOOD IN DROPS PER MINUTE BEFORE FIRST HEMORRHAGE	VOLUME-FLOW OF BLOOD IN DROPS PER MINUTE AFTER FIRST HEMORRHAGE	PULSE RATE BEFORE FIRST HEMORRHAGE	PULSE RATE AFTER FIRST HEMORRHAGE	MEAN BLOOD PRESSURE IN MM. HG. BEFORE FIRST HEMORRHAGE	MEAN BLOOD PRESSURE IN MM. HG. AFTER FIRST HEMORRHAGE
1	1.02	15.7	10.4	112	182	100	92
2	1.03	62.0	46.0	84	180	124	124
3	0.95	20.3	17.2	132	150	98	92
4	0.76	89.0	65.5	98	108	114	90
5	1.00	66.3	43.0	108	114	92	73
6	0.68	60.0	45.0	68	82	124	132
7	0.98	55.5	50.0	82	98	139	137
8	0.68	37.0	33.4	66	70	116	113
9	0.68	129.0	128.0	104	236	118	131
10	1.05	37.0	35.7	103	123	83	89

the animal and it is interesting to note in this connection that the hemorrhage which elicited the smallest decrease in the volume-flow of blood produced the greatest increase in the pulse rate. The effect of initial hemorrhage on the mean blood pressure was also variable. In four experiments the mean blood pressure remained constant or increased, in two only a slight fall in pressure occurred and in four a definite fall.

Analysis of the tables for experiments 1, 8, 9 and 10 in which gum-saline was injected shows that the volume-flow of blood in all cases was increased in greater proportion than the corresponding increase in blood-volume.

SUMMARY AND CONCLUSIONS

The effects of graded hemorrhage and subsequent intravenous injection of gum-saline solution (6 per cent gum arabic in 0.9 per cent sodium chloride solution) on the volume-flow of blood through the striated muscle of the dog was studied.

Hemorrhage with one exception invariably produced a decrease in the volume-flow of blood. On the whole the hemorrhages occurring later in the experiments had a greater effect upon the volume-flow of blood and mean blood pressure than the initial hemorrhages, invariably decreasing the volume-flow of blood in disproportion to the decrease in blood-volume.

The effects of initial hemorrhage on volume-flow of blood were more variable, ranging from a decrease in volume-flow of blood less or equal to the reduction in blood-volume to one far in excess of the reduction in blood-volume.

In experiments in which the injection of gum-saline solution proved non-toxic, injection increased the volume-flow of blood out of proportion to the increased blood-volume. From this it follows that if *nutrient-flow* (concentration of blood constituents × volume-flow of blood) is of significance, infusion of inert solution after partial depletion of blood-volume should theoretically improve the nutrition of striated muscle. Since striated muscle forms the bulk of the mammalian organism, infusion of inert solution after depletion of the blood-volume might improve the condition of the organism as a whole.

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ON THE RELATION OF BLOOD-VOLUME TO TISSUE-NUTRITION

III. The Effects of Hemorrhage and Subsequent Intravenous Injection of Gum-Saline Solution on the Response of Striated Muscle to Rapid Stimulation with Supplementary Data on the Effects of Hemorrhage and Infusion on Salivary Secretion Elicited by Intravenous Injection of Pilocarpin

ROBERT GESELL assisted by C. A. MOYLE

From the Department of Physiology of the University of California, Berkeley

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In the experiments described in the preceding paper (1) it was found that hemorrhage and infusion of gum-saline solution affected the volume-flow of blood through the striated muscle of the dog in a way similar to that reported for the submaxillary gland (2); hemorrhage as a rule decreasing the volume-flow of blood out of proportion to the decrease in blood-volume and infusion increasing the volume-flow out of proportion to the increase in blood-volume. Thus it follows that hemorrhage decreased, and infusion of an inert solution increased, the nutrient-flow.

The object of the present experiments was to demonstrate by direct experiment whether or not such changes in nutrient-flow elicited by hemorrhage and subsequent infusion of gum-saline solution affect the response of the muscle to rhythmic stimulation.

Method. Contractions of the sartorius muscle of the dog were studied. One-half to three-quarters of an hour prior to the experiment 0.4 cc. of a 2 per cent solution of morphine sulphate was injected hypodermically, and this anesthesia was later supplemented with uniform ether anesthesia. The skin was incised longitudinally over the lower third of the sartorius, the edges and under surface of the distal end freed from the neighboring tissues, and the insertion on the bone severed with minimum interference with the circulation of the muscle. The

[1] Preliminary report of this work will be found in the Proceedings of the Society for Experimental Biology and Medicine, 1921, vol. xix, p. 56.

tendon was next attached by a system of pulleys to an ordinary muscle lever, the tension on which was regulated by means of an adjustable spiral steel spring. The muscle was excited by means of a specially constructed stimulating electrode inserted transversally under the muscle at the junction of the lower and middle thirds. This electrode consisted of a flat piece of hard rubber about 4 cm. long, 1.8 cm. wide and 2 mm. thick, mounted on which were two slightly elevated strips of platinum running longitudinally and separated approximately 10 mm. Rhythmic break shocks from an inductorium were delivered by an electrically driven stimulus selector. The bulk of the exposed muscle was re-covered with its overlying skin during observations to prevent exposure and cooling. Mean blood pressure was recorded with the mercury manometer. Time was recorded in seconds.

Two procedures were followed in the study of the effects of hemorrhage and of infusion of gum-saline solution on muscular contraction. In one the muscle was stimulated equal periods of time at a rate producing, or not quite producing, complete tetanus, these periods being interrupted, with some few exceptions, with equal periods of rest. (See plate 3.) Having elicited at least two myograms similar in contour and suitable for controls, two more sets of contractions elicited by similar stimulation after similar periods of rest were recorded immediately after hemorrhage, these records in turn being followed by myograms elicited after intravenous injection of gum-saline solution. In the second procedure long-continued uninterrupted stimulation at a constant but slower rate was employed. (See plates 1 and 2.) The rate was such that tetanic contraction was never approached. If marked fatigue occurred the rate of stimulation was adjusted so that a fairly constant fatigue-level was ultimately established. After having obtained such a fatigue-level, the animal was bled and later injected without cessation of stimulation. In the two procedures just described, the height and amplitude of contraction were used as indices to the effects of changes in blood-volume resulting from hemorrhage and infusion.

While in general these two distinct procedures were followed throughout, yet in each experiment there were variations in sundry details such as the exact rate of stimulation, the duration of the periods of stimulation, the duration of the periods of rest, the tension in the spring attached to the muscle lever against which the muscle contracted, the cross sectional area of the muscle, the amount of hemorrhage and the amount of injection. These variables were not accurately con-

trolled, for we made no effort at a quantitative study of the exact amount of mechanical and chemical energy liberated with contraction in relation to the cross sectional area or mass of muscle tissue. We confined our efforts for the time to a less quantitative study of the significance of nutrient-flow in relation to muscular contraction. The main details concerning us were the amount of hemorrhage and the amount of injection associated with a definite change in response of the muscle to rapid stimulation.

RESULTS. Eight experiments were performed on the sartorius muscle, the data of which are shown in the tables and in plates 1 to 4. Supplementary data from one experiment on the relation of nutrient-flow to salivary secretion are shown in plate 5.

Experiment 1. The table shows the hemorrhages and injections of gum-saline solutions in per cent of body weight. Observation 3 of this experiment is reproduced in figure 1, plate 1, the first graph being a myogram elicited before hemorrhage, the second after hemorrhage (1.50 per cent of the body weight) and the third after subsequent injection of gum saline solution (1.75 per cent of the body weight).

TABLE 1

Hemorrhages and injections in experiment 1. 9/16/21

Weight of dog 8.00 kgm.

OBSERVATION	HEMORRHAGE IN PER CENT OF BODY WEIGHT	INJECTION IN PER CENT OF BODY WEIGHT
1	2.12	2.12
2	1.50	1.50
3	1.50	1.75

Experiment 2. In this experiment the effects of hemorrhage and injection on the response of the muscle to continuous stimulation were demonstrated. (See figure 2.) The time at which hemorrhage (0.52 per cent of the body weight) and injection (1.04 per cent of the body weight) occurred is discernible on the blood pressure record. Perhaps it should be mentioned here that these results were obtained near the close of the experiment after a succession of hemorrhages (14) and injections (20) amounting to 9.65 and 11.39 per cent of the body weight respectively, carried out for the purpose of repeating a fortuitous observation on the effects of blood-volume on muscle tonus.[2] These hemorrhages and injections undoubtedly led to a marked dilution of

[2] See note, p. 427.

the blood up to the time at which the myogram in figure 2 was elicited. The particular hemorrhage employed in this observation must therefore have reduced the nutrient-flow to a relatively low value, which may possibly account for the marked effects on fatigue, yet in some of the following experiments small initial hemorrhages affected the muscular response as well.

Experiment 3. Figure 3 reproduces observations 13 and 14 in which continuous stimulation was employed. The irregularities seen on the myogram were due to difficulties with the stimulus selector encountered throughout the greater part of this experiment and interfering with the demonstration of effects of changes in blood-volume before observations 9 and 10. Observations 9 and 10 show results comparable to those in observations 13 and 14.

TABLE 2

Hemorrhages and injections in experiment 3. 9/26/21

Weight of dog 8.75 kgm.

OBSERVATION	HEMORRHAGE IN PER CENT OF BODY WEIGHT	INJECTION IN PER CENT OF BODY WEIGHT
1	0.57	
2	0.59	
3	0.41	
4		0.57
5	1.97	0.57
6	0.96	0.57
7		2.86
8	1.44	1.71
9	1.03	
10	0.68	0.68
11		1.03
12		0.86
13	1.07	
14	0.48	1.55

Experiment 4. No effects of the initial hemorrhage and injection were observed in this experiment, probably due to the fact that the continuous stimulation which was employed at the outset was too rapid and elicited a progressive fatigue. The effects of hemorrhage and injection following the initial observation are seen in figure 4a, observations 4, 5 and 6 and in figures 4b, c and d, observation 7.

TABLE 3

Hemorrhages and injections in experiment 4. 9/28/21

Weight of dog 11.00 kgm.

OBSERVATION	HEMORRHAGE IN PER CENT OF BODY WEIGHT	INJECTION IN PER CENT OF BODY WEIGHT
1	1.00	0.45
2		0.91
3		0.45
4	1.11	
5	0.64	0.64
6		0.91
7	1.02	1.09

Experiment 5. In this experiment the initial hemorrhage and the initial injection as well as the later hemorrhages and injections exerted a definite effect upon the response of the sartorius muscle. Observations 4, 5 and 6 are shown in figures 5a, b and c, 5d and 5e respectively.

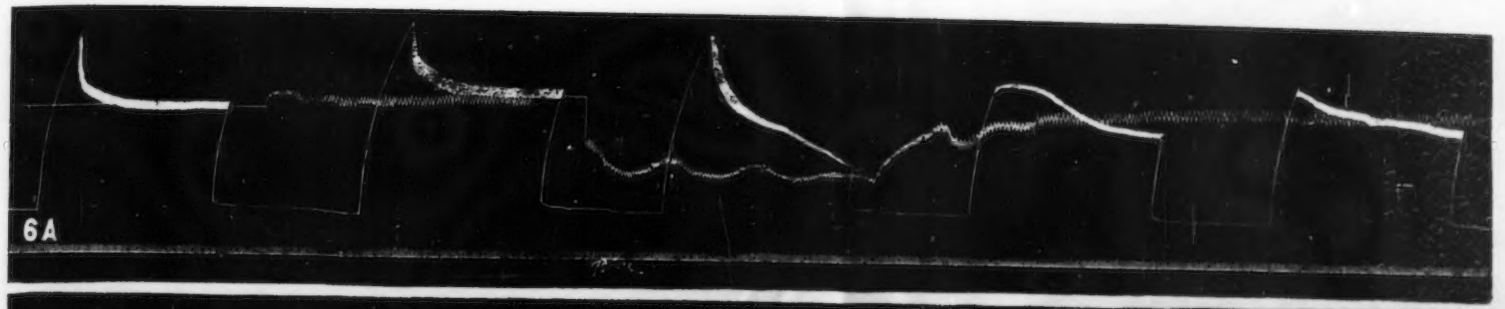
TABLE 4

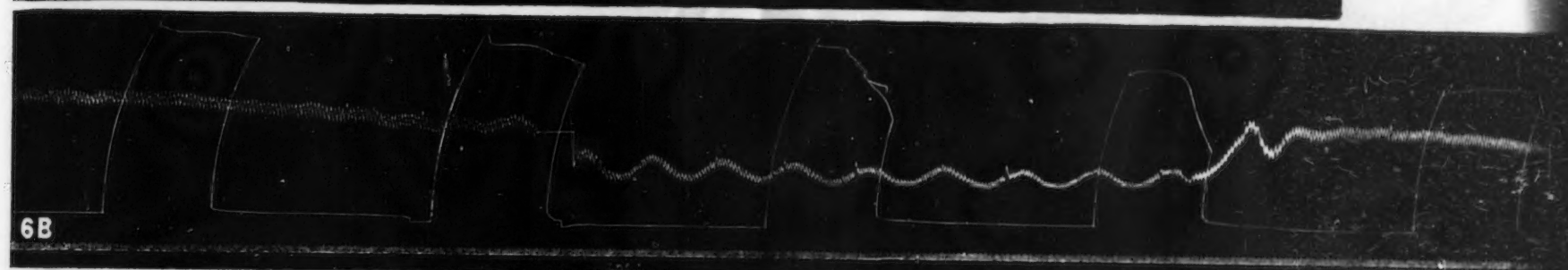
Hemorrhages and injections in experiment 5. 9/29/21

Weight of dog 10.85 kgm.

OBSERVATION	HEMORRHAGE IN PER CENT OF BODY WEIGHT	INJECTION IN PER CENT OF BODY WEIGHT
1	1.01	
2	0.48	1.49
3	0.98	1.34
4	1.01	1.20
5	1.01	1.29
6	1.03	2.12

Experiment 6. This experiment showed striking effects of hemorrhage and injection of gum-saline solution, perhaps due to the fact that relatively small hemorrhages produced marked circulatory disturbances in this animal as indicated by the fall in mean blood pressure. Figure 6a shows the effects of hemorrhage amounting to 1.00 per cent of the body weight and subsequent injection amounting to 1.20 per cent of the body weight. Figure 6b shows the effects of later hemorrhage and injection amounting to 0.85 per cent and 1.10 per cent of the body weight respectively.





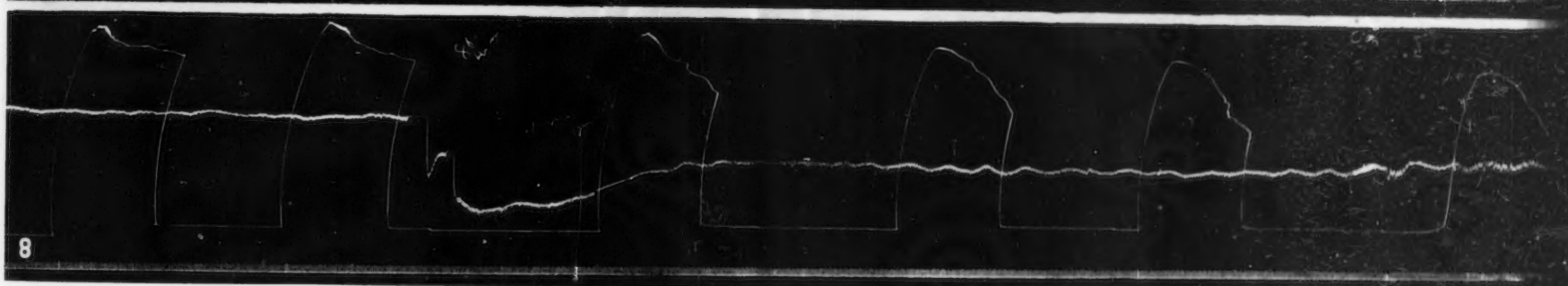
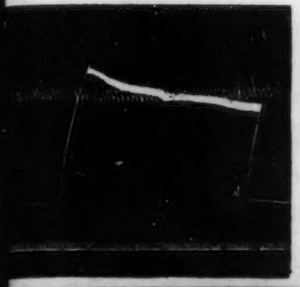
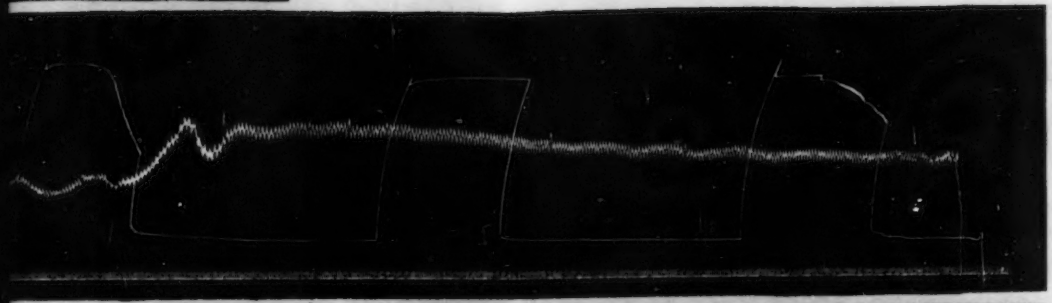
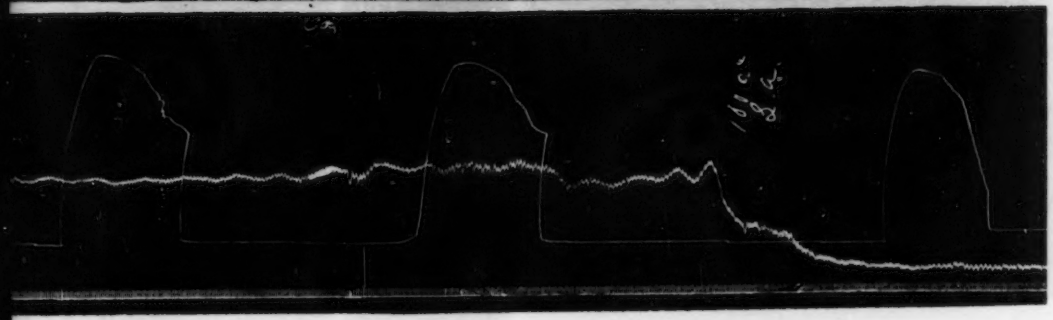


Plate 3
Blood-volume and tissue-nutrition series







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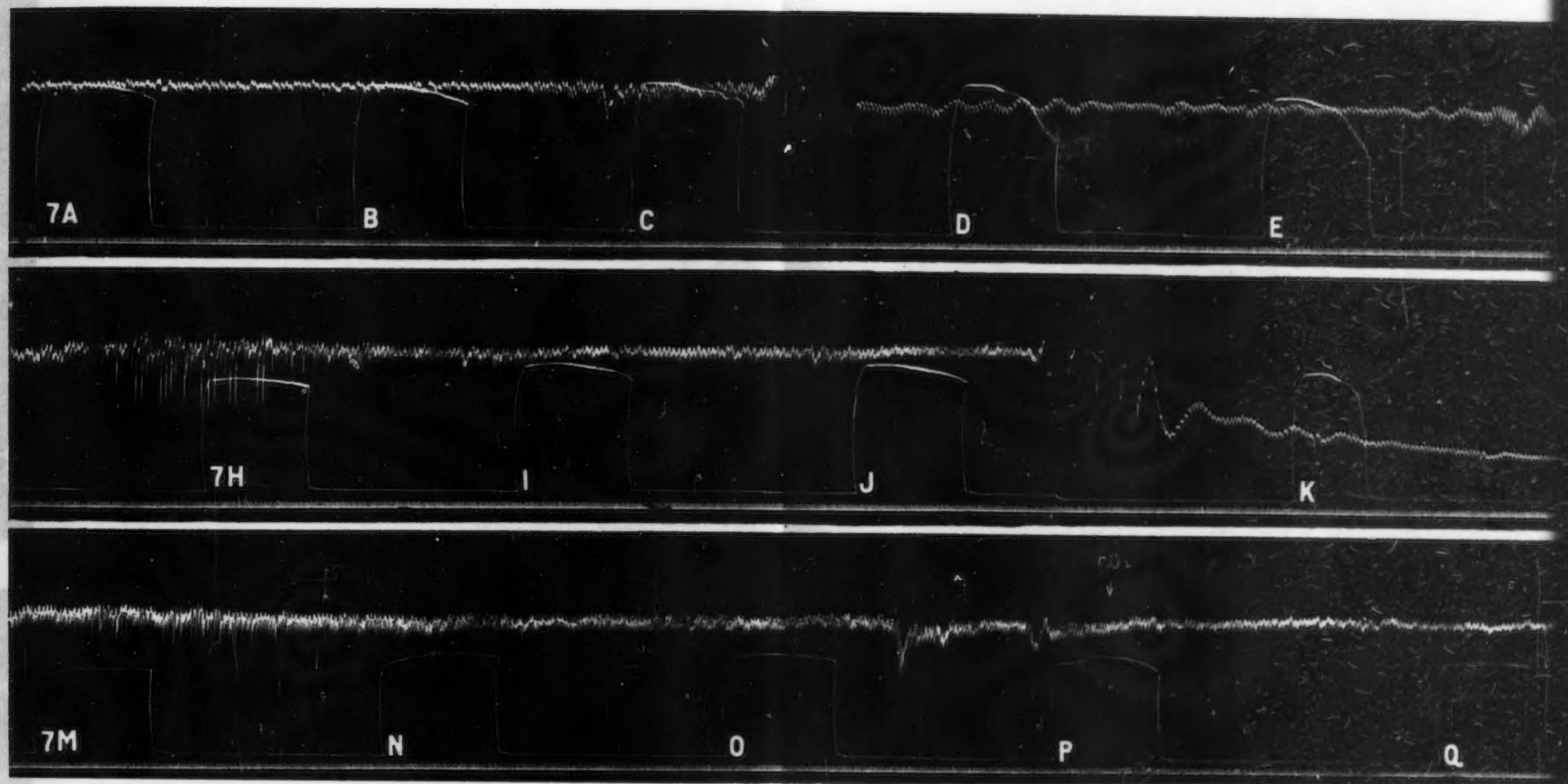
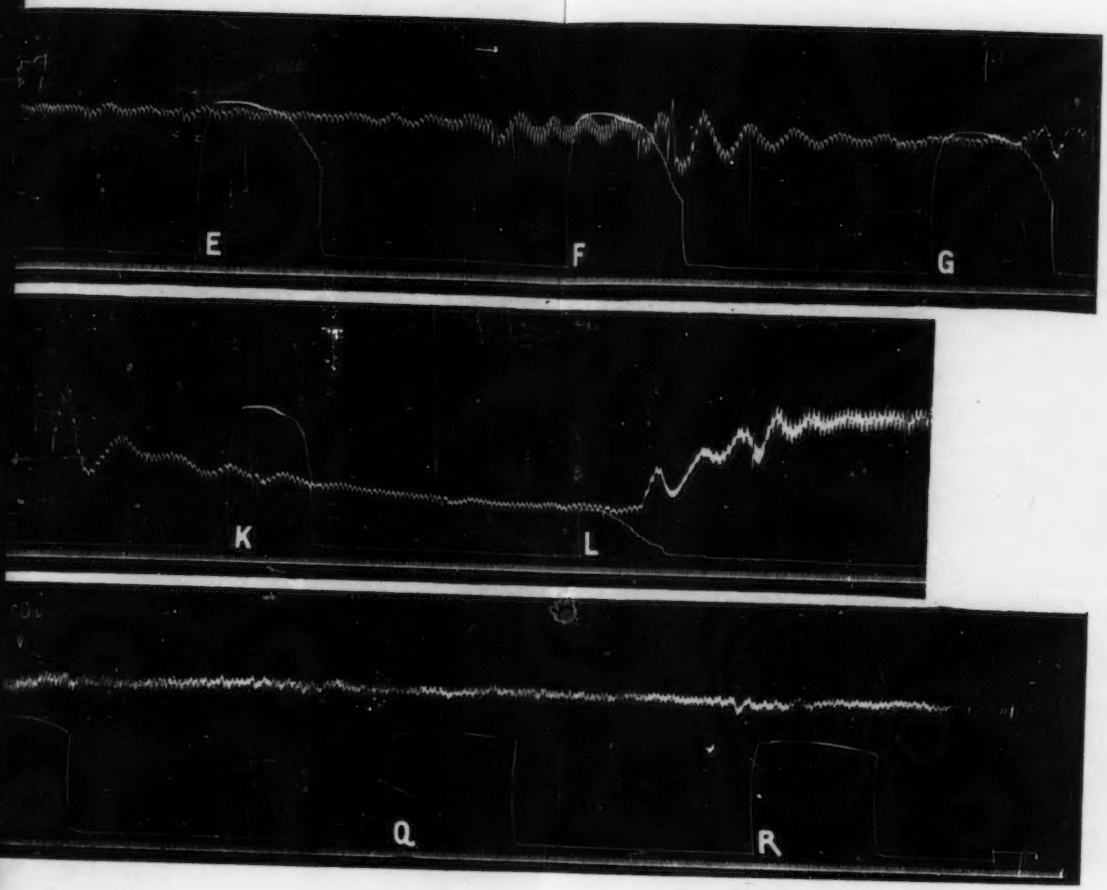


Plate 4
Blood-volume and tissue-nutrition series



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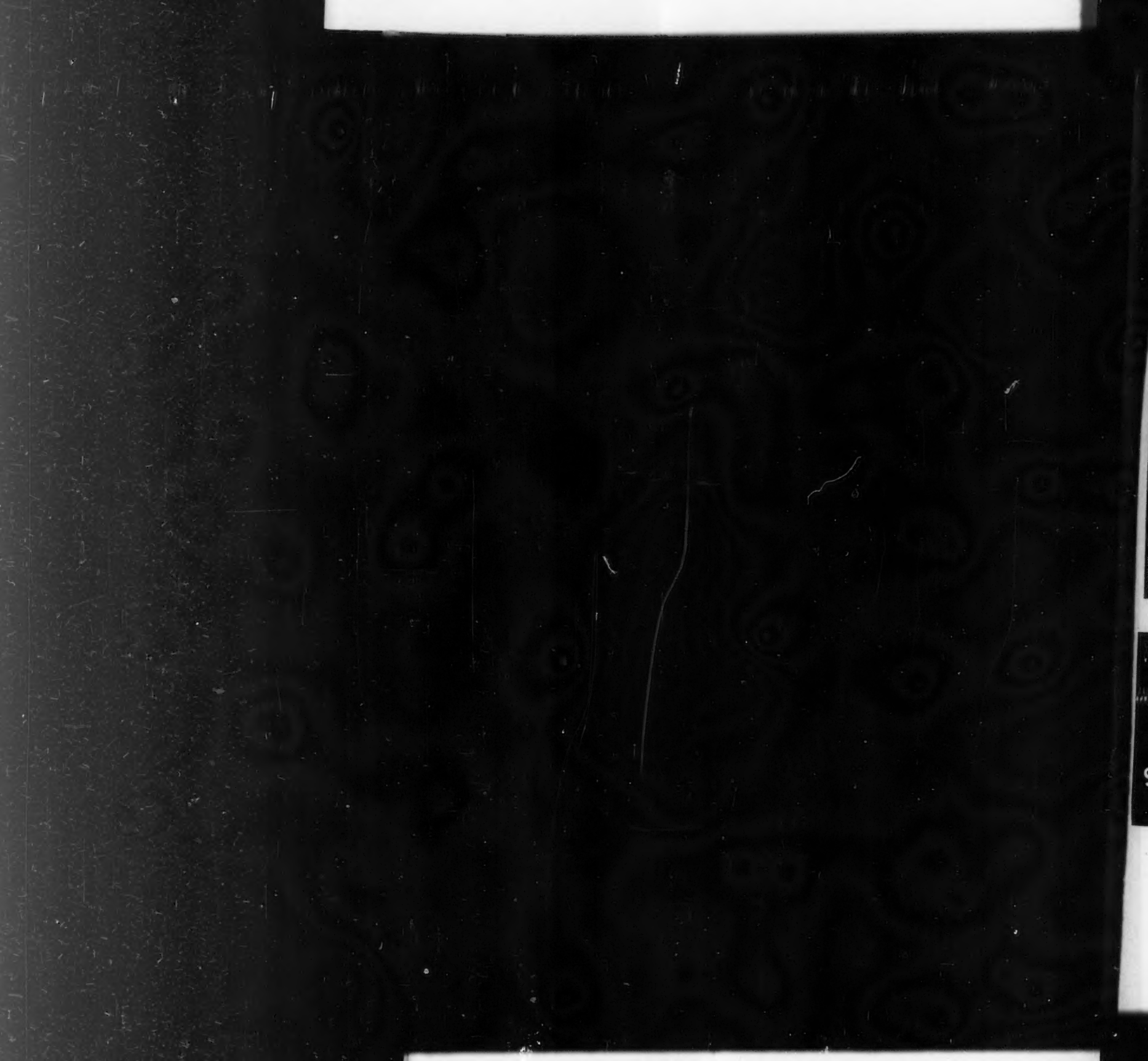


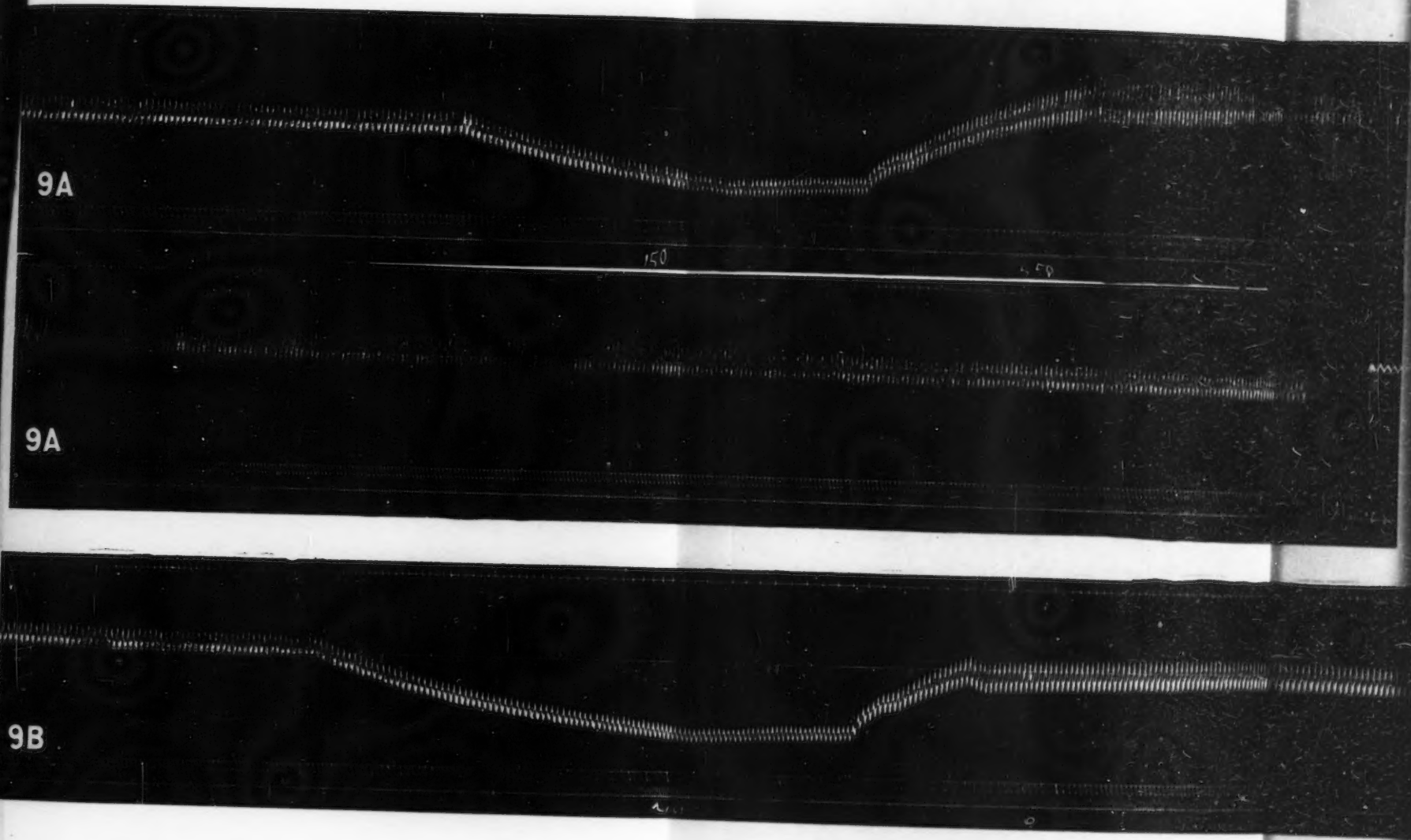
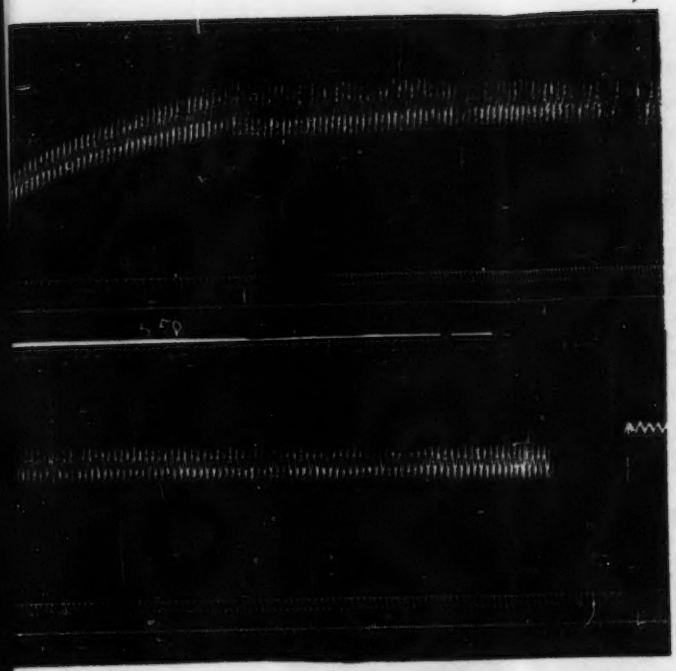


Plate 5
Blood-volume and tissue-nutrition series



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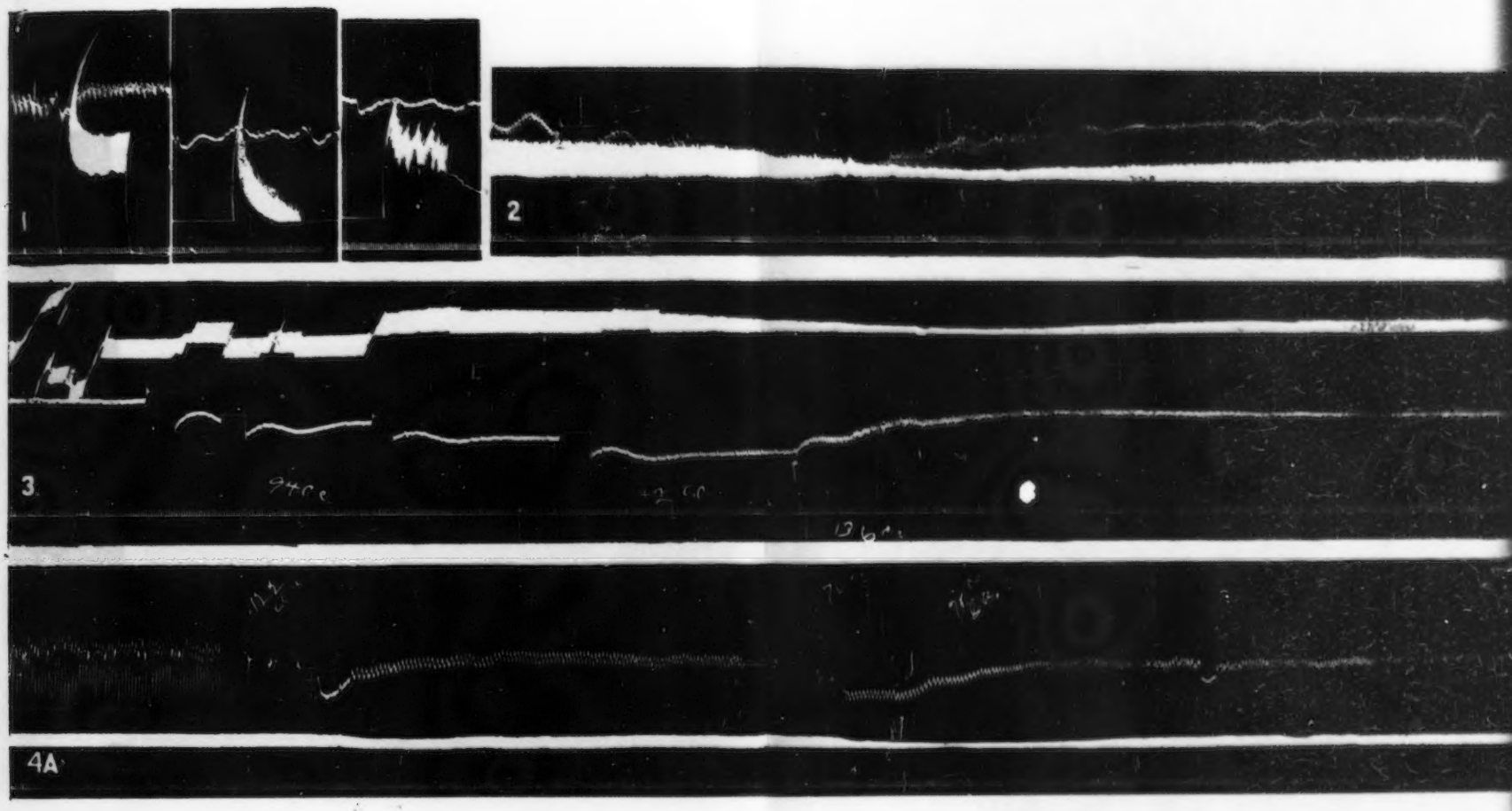
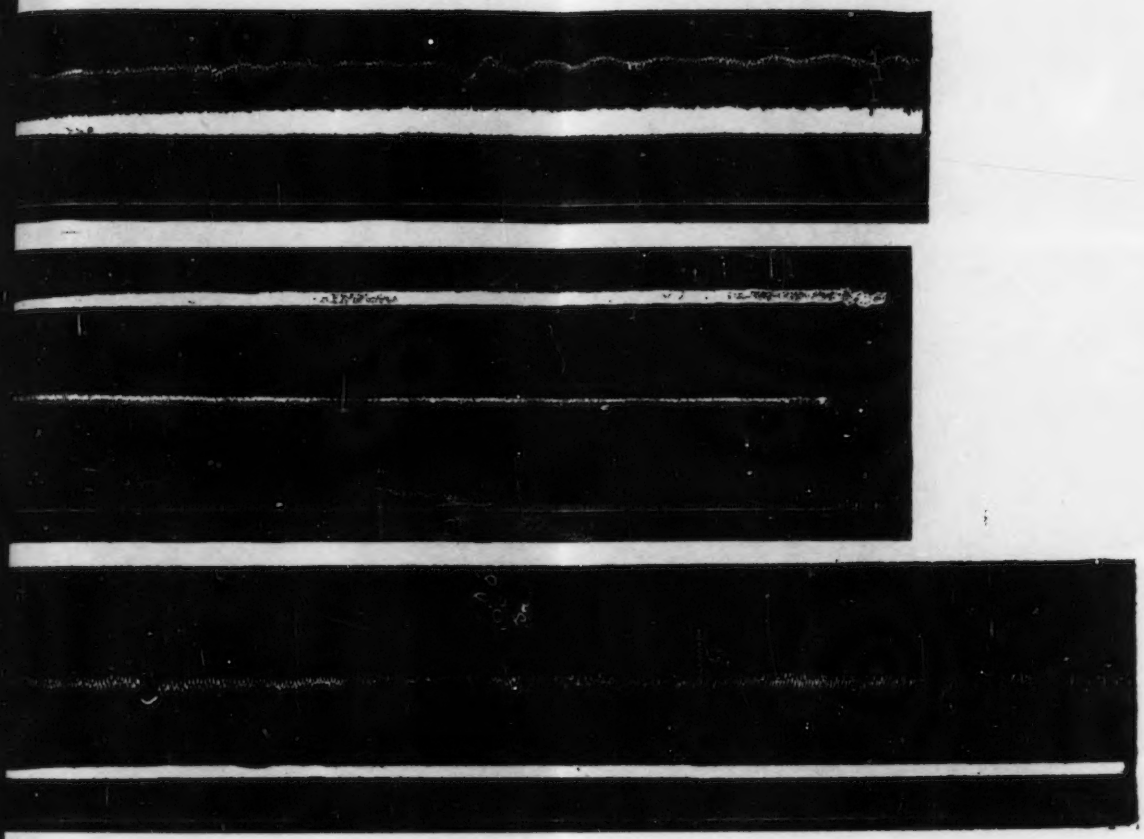


Plate 1
Blood-volume and tissue-nutrition series



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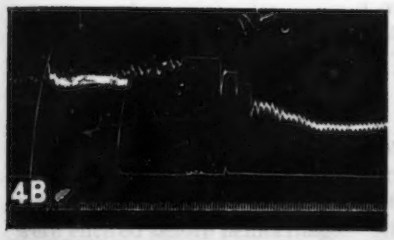
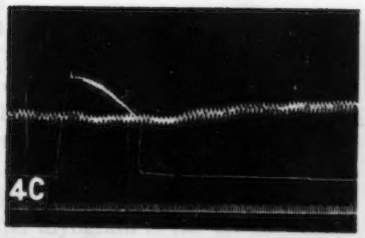
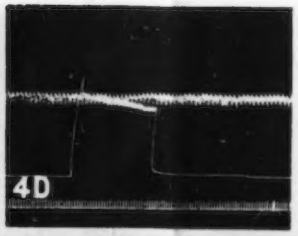
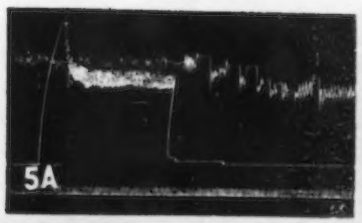








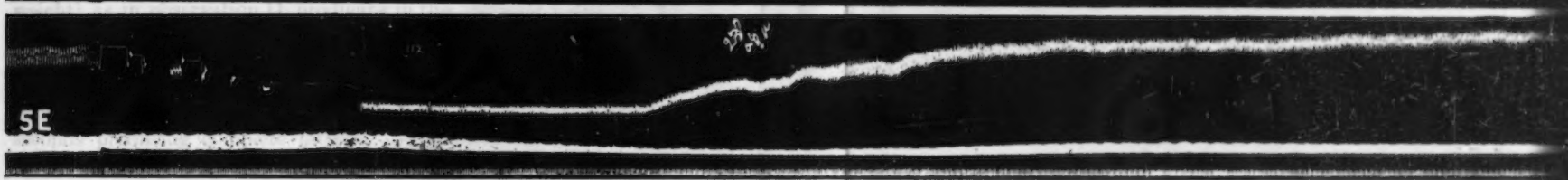
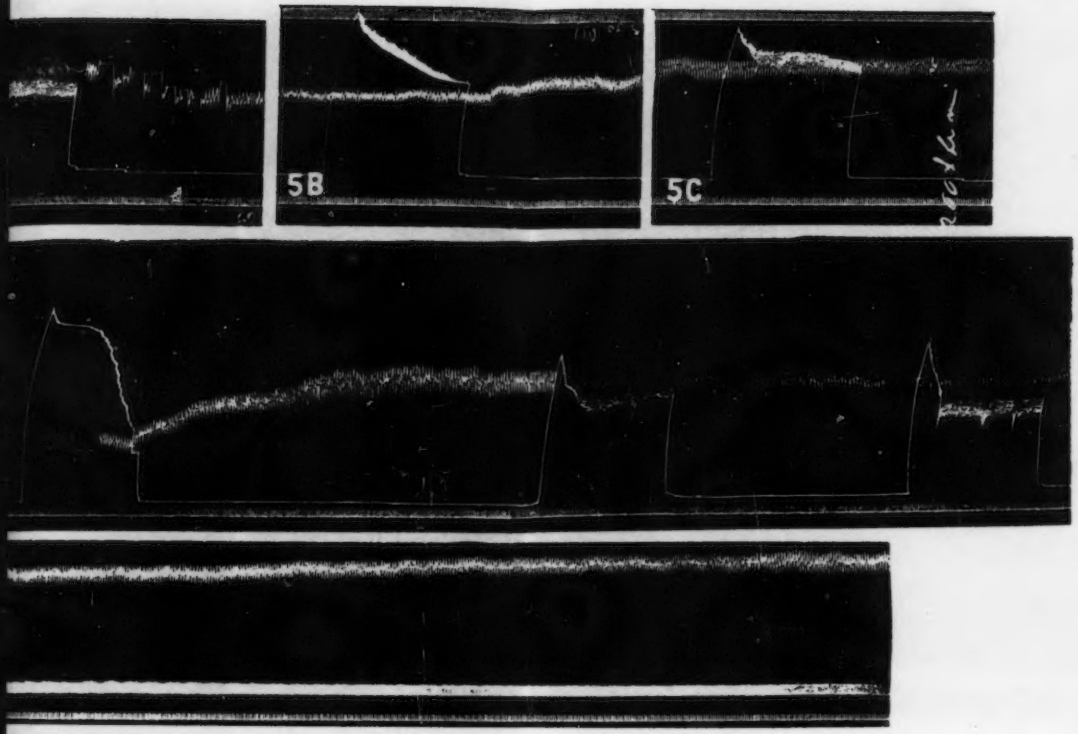


Plate 2
Blood-volume and tissue nutrition series



trition series

TABLE 5

Hemorrhages and injections in experiment 6. 9/30/21

Weight of dog 7.00 kgm.

OBSERVATION	HEMORRHAGE IN PER CENT OF BODY WEIGHT	INJECTION IN PER CENT OF BODY WEIGHT
1	1.00	1.20
2	0.85	1.10

Experiment 7. Figure 7 shows a continuous series of myograms of 60 seconds duration each, separated by intervals of rest lasting in most cases about 2 minutes. The effects of respiring a mixture of 3.5 per cent of carbon dioxide in room air as well as the effects of hemorrhage and injection were recorded. The first three myograms, A, B and C, were elicited before hemorrhage. After myogram C the initial hemorrhage (0.99 per cent of the body weight) occurred. Though this hemorrhage produced only a very slight fall in the mean blood pressure, it had marked effects on the ability of the muscle to respond to rhythmical stimulation, as is seen in myograms D and E. Carbon dioxide was administered prior to and during myogram F. The harmful effects of the gas are noticed by comparing myogram F with myograms E and G. Myograms H, I and J show the beneficial effects of replacement of lost blood with gum-saline solution. Note that recovery is not immediate, that myograms I and J are higher than myogram H. After myogram J double the amount of blood was drawn (2.0 per cent of the body weight) as in observation D, producing in this instance a distinct fall in blood pressure. On account of the development of very marked fatigue following this hemorrhage, stimulation was continued only 40 instead of 60 seconds. The recovery of the muscle after myogram K is very slight, the second series of stimuli (L) also lasting only 40 seconds producing complete fatigue. After myogram L gum-saline (2.44 per cent of the body weight) was injected. The usual recovery occurred as shown by myograms M, N and O. In myogram P the effects of administering carbon dioxide are seen again. In this instance carbon dioxide was administered while the blood-volume was normal.

TABLE 6

Hemorrhages and injections in experiment 7. 10/5/21

Weight of dog 9.50 kgm.

OBSERVATION	HEMORRHAGE IN PER CENT OF BODY WEIGHT	INJECTION IN PER CENT OF BODY WEIGHT
1	0.99	1.05
2	2.00	2.44

Experiment 8. The effects of the initial hemorrhage amounting to 0.98 per cent of the body weight are shown in figure 8. Injection of gum-saline to make up this lost blood proved toxic, producing a very large fall in blood pressure and a rapid onset of fatigue on stimulation.

Experiment 9. Bayliss has reported the effects of hemorrhage and infusion on salivary secretion elicited by intravenous injection of pilocarpin. Having at our command a bloodless method of recording the volume-flow of blood, we attempted to repeat this observation in one experiment on the submaxillary gland of the dog. The results were so striking that we publish herewith two of several records obtained. In each record the tracings from above down represent drops of saliva, mean blood pressure, volume-flow of blood and time in seconds. The lower record which is the first of the two sets of observations shows the effects of hemorrhage amounting to 1.35 per cent of the body weight and an injection of 1.65 per cent of the body weight. In the upper record the effects of a smaller hemorrhage (0.95 per cent of the body weight) and a very much larger injection (2.85 per cent of the body weight) are shown. Hemorrhage here as in the other graph elicited a decreased flow of blood and secretion. The large injection increasing the blood-volume considerably above that obtaining before hemorrhage elicited a correspondingly large volume-flow greater than the prehemorrhage flow. This rapid flow of blood was accompanied by a flow of saliva considerably faster than that obtaining before hemorrhage.

SUMMARY AND CONCLUSIONS

The effects of hemorrhage and subsequent intravenous injections of gum-saline solution on the response of the sartorius muscle of the dog to rapid stimulation with the induced current were studied.

It was found in general that hemorrhage decreased the ability of the muscle to respond to stimulation and that injection of gum-saline solution improved the response. In one instance hemorrhage amounting to 0.99 per cent of the body weight and having little effect upon the mean blood pressure exerted a decidedly detrimental effect upon muscular contraction. This effect was largely removed by later replacement of blood with gum-saline solution.

Hemorrhage and injection had corresponding inhibitory and accelerating effects upon salivary secretion elicited by the intravenous injection of pilocarpin. Injection of gum-saline solution in amount considerably greater than the hemorrhage increased the volume-flow of blood and the flow of saliva considerably above the prehemorrhage flows.

We feel that the experiments indicate that augmented metabolism of striated muscle is closely dependent upon nutrient-flow; that the decreased response of muscle after hemorrhage is due in large part to the decrease in flow of nutrient material to the tissues, and a decrease in flow of constituents which neutralize and carry away the metabolites (CO_2 carriers, e.g.); that the improved response of muscle after the injection of gum-saline is due in large part to the markedly accelerated flow of a relatively slightly diluted blood (increased nutrient-flow). The possibility, however, of decreased and increased capillary pressures playing a part in the nutrient condition of the tissue as a result of changes in the rate of formation and rate of flow of lymph in the tissue must be considered. Perhaps this factor of lymph formation is of greater significance in secreting tissues, such as the submaxillary gland, where water and other constituents of the blood are liberated with tissue activity, than in striated muscle whose prime function is the liberation of contractile and thermal energy.

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NOTE: Perhaps a note on the effects of hemorrhage and injection on the length of muscle might not be amiss here in relation to the observation which we made in the first paper of this series, namely, that in the unanesthetized animal hemorrhage produced a palpable softening of the voluntary muscles. In the experiment to which this note pertains hemorrhage produced a lengthening of the resting muscle and also a lengthening, that is, a lowering of the line joining the ends of the periods of relaxation in the rhythmically contracting muscle. (See fig. 10.) This effect of hemorrhage was not apparent in any of the other 7 experiments. We therefore do not wish to press without further experimentation the possibility previously suggested that the reduced consumption of oxygen following hemorrhage in the unanest etized dog is dependent upon a loss of muscular tonus.

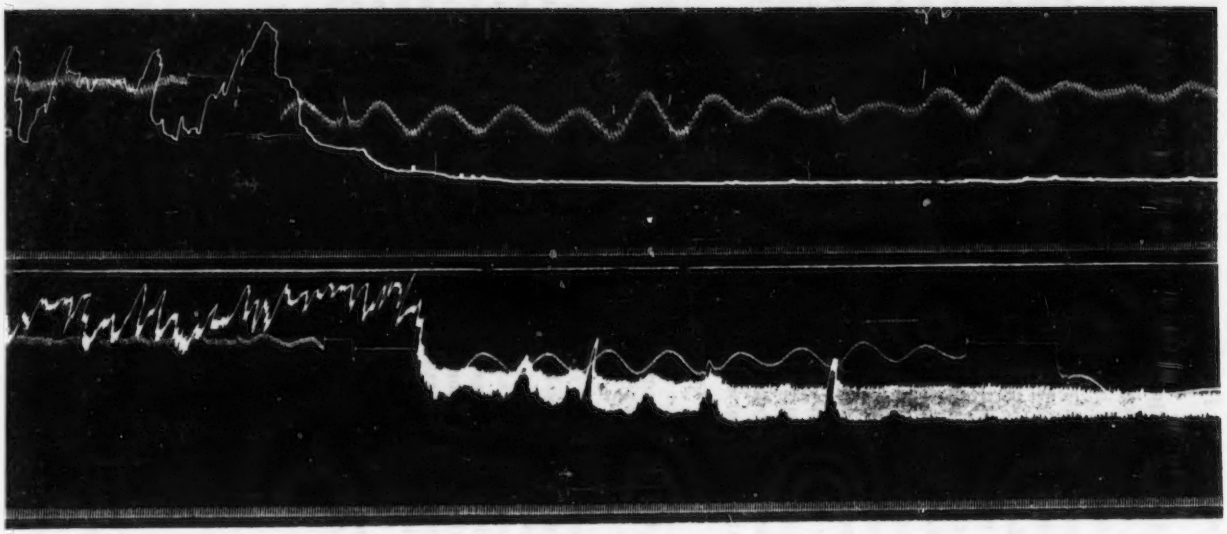

Fig. 10

A STATISTICAL STUDY OF THE PULSE RATE AND THE ARTERIAL BLOOD PRESSURES IN RECUMBENCY, STANDING, AND AFTER A STANDARD EXERCISE

EDWARD C. SCHNEIDER AND DOROTHY TRUESDELL

From the Medical Research Laboratory and School for Flight Surgeons, Mitchel Field, Long Island, New York

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In attempting to find well-founded conclusions regarding normal circulatory conditions, with the expectation of using cardio-vascular reactions for estimating the physical fitness of men, it soon became evident that a statistical investigation of postural and exercise reactions might still contribute something of value to our knowledge of commonly used circulatory factors.

For this purpose the pulse rate and the three arterial pressures—systolic, diastolic and pulse pressures—have been determined under four conditions: viz., recumbency, standing, immediately after a standard exercise and 2 minutes after the exercise. The usual procedure was to require the subject to recline for 5 minutes and then to count the pulse rate, for 20 or 30 seconds, the count being continued until two consecutive intervals gave the same result. After this the arterial blood pressures were determined. The subject was next requested to stand and be at ease for 1 or 2 minutes to allow the pulse to assume a uniform rate. When two consecutive counts of the pulse rate were the same, this rate was recorded and the arterial pressures for the standing position then determined. It was always the custom to verify the blood pressure readings. The subject next stepped on a chair about 18 inches high, five times in 15 seconds, this exercise being timed with a watch. Immediately both feet were on the floor after taking the exercise, the pulse rate was counted for 15 seconds and after that the arterial pressures were determined. Following this the subject stood at ease for the remainder of a 2-minute period, when the pulse rate and arterial pressures were again determined.

It was not always possible to guard against the influence of nervousness but an attempt was made to do so. As soon as the subject had

reclined the sphygmomanometer cuff was put on and this was inflated and deflated several times, or preliminary determinations were made of the arterial pressure and as a rule a conversation regarding laboratory subjects of general interest was kept up in an attempt to divert the mind of the subject from the observations being made. So it is believed that the results are as near normal as can be obtained on any group of men except those trained to serve as subjects of experimental study.

The greater part of the investigation deals with 2000 aviators, whose ages ranged between 18 and 42 years, the arithmetical mean age being 24.8 ± 0.05. In order to have a check on these, 200 additional unselected cases were even more carefully examined and compared with the large group. In addition, a study was made of two small groups of men, judged by clinicians at the time of examination to be physically well and fit. The first group was examined in 1919 and 1920 and comprised 144 cases, and the second included 204 aviators who were participating in a special experimental bombing campaign during the summer of 1921 at Langley Field.

The data will be discussed from the standpoint of the distribution of cases, the amount of postural and exercise change, time required to return to normal after exercise and the interdependence of the several factors. To determine the extent that one circulatory factor may be dependent upon others, two methods will be used. In the first coefficients of correlation will be considered and in the second a selection of groups of cases showing extreme and opposite conditions with respect to one factor will be examined as to the mean condition and reaction of all the other circulatory factors. Throughout the entire paper lines of means will be used to indicate circulatory tendencies and relationships.

THE FREQUENCY OF THE HEART BEAT. In 1796 Falconer (15) pointed out that the frequency of the pulse is greater in standing than in the sitting or lying postures. The greatest difference reported by him was 13, the least 1, and the average 6.33 beats. In 1830 Graves (18) also showed that in healthy persons the pulse was from 6 to 15 beats more frequent in the erect than in the horizontal position, the difference being least in persons who had a slow rate. He found the greatest difference, ranging from 30 to 50 beats, in cases of fever. Guy (19) found in 100 young men of an average age of 27 years that the average was 78.9 standing, 70.1 sitting and 66.6 lying. For 50 young women of the same age the average was 89.3 standing, 82 sitting and 80.2 lying. He also found that the effect of posture varied with the frequency of the pulse, the changes being least in men with a slow rate.

Volkmann (43), in 1850, showed the influence on pulse frequency of such factors as age, sex, size, taking of food, temperature, time of day, condition of rest or activity and emotions. He found that the frequency of the heart beat decreases until the 20th year, then maintains a level until 55 years of age, after which a gradual rise occurs with the advance of years. He and others found that the height of the person exerts an influence, the frequency being less in tall than in short persons. The attempts to formulate a mathematical formula to express the relationship have not been satisfactory. Knox (27), in 1815, showed that the pulse is more rapid in the morning than in the evening. In the morning the change of posture from recumbency to standing increased the frequency by about 15 to 20 beats, at midday about 10 and in the evening 4 to 6 beats. He also reported that the heart is more excitable in the morning, that food and exercise accelerate its action to a greater degree in the early part of the day than in the evening. While it is generally recognized that rest diminishes the pulse frequency, Klewitz (26) finds that during day sleep the rate is substantially higher than during night sleep.

Guy reported that the difference between the standing and lying pulse ranges from 0 to 44, averaging 10 beats for males under 20 years of age, and 14 beats for those above that age. Geigel (17) observed a similar age difference.

Pulse rate in recumbency. The pulse rate of the reclining position ranged for our 2000 cases between 42 and 117, and for the 200 cases from 48 to 102, the smaller group having proportionately fewer exceptional cases. The distribution of the 2000 cases has been plotted in figure 1. The mode for this curve, as well as that of the 200 cases, is at 72. The arithmetical mean is 74.11 $\pm$ 0.15 for the larger, and 75.21 $\pm$ 0.47 for the smaller group. These averages are somewhat under 77, as given by Geigel for the general average. The similarity of distribution in the two groups is further brought out by noting the percentage of cases that occur under or above certain rates. Thus in the group of 2000 cases there were in recumbency 71.5 per cent with a rate under 80, 35.9 per cent with 70 or less, and 10.5 per cent with 60 or less; while in the group of 200 there were 67 per cent under 80, 31 per cent with 70 or less, and 7.5 per cent with 60 or less. Among the 2000 cases there were 8.5 per cent and in the small group 11 per cent which showed a rate of 90 or more.

It has long been recognized that in athletic persons the pulse rate is slower than the general average and that the same is true, though in

lesser degree, for those in very good health. Meylan (31) regards a recumbent posture pulse rate between 40 and 80 as one of the favorable health signs. Parkinson (34) found that the average rate for 20 healthy soldiers was 72. McCurdy (30) found that the general average for boys of post-pubescent age was 65.9; for athletic boys 61.5; and for newsboys, who covered 3 to 9 miles each morning, 60.7. Our two groups, judged to be in excellent condition by clinicians, more nearly resemble these conditions than the two groups of unselected cases. The arithmetical mean was 72.1 ± 0.5 for our group of 144 examined in 1919 and 1920, and 70 ± 0.4 for the Langley Field group of 204. The recumbent posture rates in the first ranged from 48 to 99, in the latter from 54 to 96; the mode of the frequency curve for the first was at 72, and for the latter at 66. There were 81.9 per cent in the first and 85.8 per cent in the latter group with a rate less than 80; while 4.2 per cent in the first and 2.5 per cent of the latter had a rate of 90 or more. The striking point is that none of these men had a rate of 100. It is therefore evident that men in very good health have on the average a slower reclining posture pulse rate than the general average of men.

The standing posture. The pulse frequency for the erect posture ranged between 51 and 147 in the 2000 cases and between 63 and 126 for the 200 cases. The arithmetical mean for the former group was 91.99 ± 0.19 and for the latter, 90.11 ± 0.55; while the mode of the frequency curve (see fig. 1) was at 90 for both groups. Meylan (31) considers a pulse rate between 50 and 90 for the standing position a favorable health sign. Only 50.7 per cent of the 2000 cases came between 50 and 90; while the smaller group had 55.5 per cent of the cases within that range. In the two groups there were respectively 15.4 and 18 per cent with a rate of less than 80. In the large group 24.6 per cent had a standing pulse rate above 100; while only 16 per cent of the men in the smaller group were above that figure. The two groups selected because of physical soundness show in general a slower pulse rate for the standing position. The mean rate for the 1919–20 group was 85.77 ± 0.54, and for the Langley Field group, 1921, 82.91 ± 0.42; the rates ranged between 63 and 111 for the former and between 60 and 99 for the latter; there were 72.9 per cent of the first and 85.3 per cent of the latter with a rate of 90 or less; while in the former there were 3.5 per cent and in the latter none with a standing rate above 100. These two physically sound groups, therefore, average in standing 6 or 7 beats below men generally and have decidedly fewer men with a rate above 100. Here as in other comparisons we find that the recumbent position does not as

clearly show the effects of lack of condition as do standing and exercise. In this respect Boney (4) found that tired, listless and easily fatigued patients often showed a normal pulse rate while lying down, but that on standing the rate very frequently was abnormally rapid.

The means for the four groups in the reclining position differ, at the most, only 5.2 beats; while in the standing position there is a difference of 9.2 beats. The entire range of reclining pulse rates as shown in the difference between highest and lowest rates was for the Langley Field group 42; for the 1919–20 selected group 51; for the 200 unselected cases 54; and for the large group 60, when a few extremes are omitted or 75 if all are included. In standing the difference between the highest and lowest pulse rates was 39 for the Langley Field group; 48 for the 1919–20 selected group; 63 for the 200 unselected cases; and 69 or 96 for the 2000 cases, the first figure when a few extremes are omitted and the latter when these are included. From these differences it is evident that standing rate differences are less in the two healthy groups than in the unselected groups which represent the general run of men; and furthermore that the standing data bring out health differences more clearly than do the reclining data.

Another fact which indicates that the reclining pulse frequency is a less helpful means of judging physical condition than the standing rate is found from a determination of the standard deviation. This is a measure of distribution which includes the middle two-thirds of all cases. On calculating the standard deviation of our 2000 cases we found that it was $\pm$ 10.2 for the reclining position. Since the mean pulse rate was 74.1 it follows that two-thirds of all the rates were between 63.9 and 84.3. For the standing posture the standard deviation was $\pm$ 12.6; therefore, since the mean frequency was 91.99, two-thirds of the cases fell between 79.4 and 104.6.

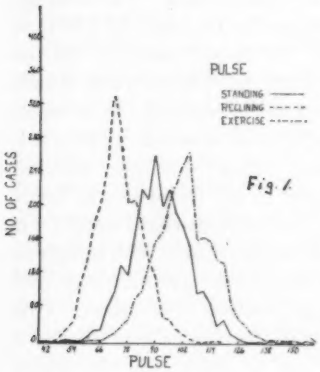
Just how rapid the pulse may be in these two postures and still be compatible with health seems not to have been determined. So far as the records are available for our cases there is nothing noted by the examining clinicians to indicate abnormality in any of the men. Furthermore, each man had undergone the usual army examination at the time of enlistment and, for at least a second time, was reëxamined as he passed through the laboratories. So we have dealt with men considered healthy by the clinician, and yet, a number of these men had a reclining rate above 100 and two reached 117; while as many as 1.05 per cent of the 2000 cases were above 100. During standing a large number had a rate above 100; while 4 were about 130, reaching 147 in one instance.

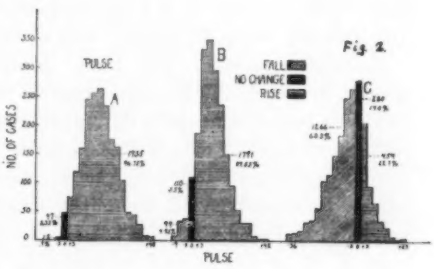
The amount of postural pulse rate change. The difference between the reclining and standing pulse rates has been the subject of frequent studies. It should be recalled that Falconer (15) in 1796 stated that the average difference is 6.33 beats, greatest 13 and least 1. Graves (18) found that for healthy persons the difference ranged from 6 to 15 beats; Schapiro (37) obtained an average of 14, with a maximum difference of 34 and a minimum of 2; Vierordt (42) found the average increase was 12 and 14 beats; Kahn (24) reported an average rise of 16 beats, maximum 28 and minimum 0. Parkinson (34) recently reported from observations on 20 athletic soldiers an average increase of 10 beats. Crampton (10) observed that in vigorous subjects the heart rate may not increase on standing, while in wearied subjects it may increase as much as 44 beats per minute. Meylan (31) believes a standing increase of not more than 16 beats is a favorable sign of physical efficiency. Geigel (17) considers that a variation of more than 30 between the lying and standing pulse rates indicates weakened heart function. He also found, from a study of 789 cases, that in 5 per cent of the men the rate was higher in the reclining than in the erect position; that in 90 per cent there was either no change or a decrease of from 1 to 30 beats; and that 5 per cent had a rate 30 or more beats less in recumbency than standing.

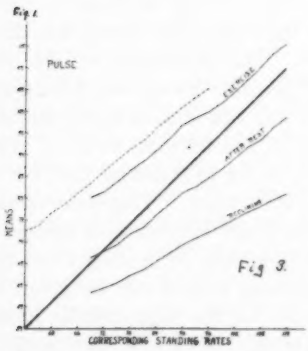
Our own groups show marked variations that help to explain the variety of differences recorded above. In the group of 2000 cases the difference between the pulse frequency for the horizontal and erect postures ranged from -15 to $+57$ There were 19 men, 0.9 per cent, who showed a more rapid pulse while reclining and 47 cases, 2.4 per cent, in which the rate was the same for both postures. The number of men who gave an unusually large increase, 30 or more beats, on standing was 252 or 12.6 per cent; of this number 26 or 1.3 per cent showed an increase of 40 or more; while in 5 or 0.3 per cent it was 50 or more. The greatest increase, 57 beats, was made by a man whose reclining rate was only 57. In figure 2*A* we have graphically shown the distribution of the 2000 cases with respect to the difference between the rates of the reclining and standing postures. The arithmetical mean for the difference between rates of the two postures was 17.9 ± 2, which is higher than that recorded by others who have studied the influence of posture on pulse frequency.

For the 200 cases, also unselected, the range of the postural differences was from -3 to $+36$. Only 2, 1 per cent, had a slower rate while standing; 4 cases, 2 per cent, showed no change on standing; while

only 10 cases, 5 per cent, gave an increase of 30 or more beats, being 36 beats in 4 cases. The average increase for the 200 cases was 14.9 ± 0.4. This is a smaller difference than that obtained for the 2000 cases because the reclining rate was greater and the standing rate less than for that group.







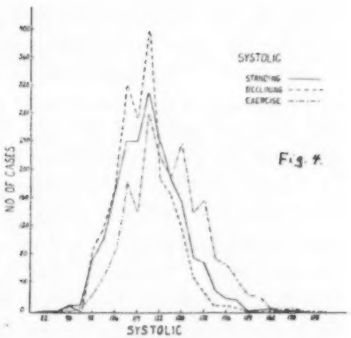


Fig. 1. Pulse rate distribution for 2000 cases.

Fig. 2. The amount of change in pulse rate. *A*, from reclining to standing; *B*, after exercise when compared with standing; *C*, 2 minutes after exercise, compared with standing.

Fig. 3. Lines of means for the pulse rate in which the heavy black line gives the corresponding standing rates. The broken line gives the standing means when the heavy black line is used to show corresponding reclining rates.

Fig. 4. The systolic arterial pressure distribution of 2000 cases.

The groups of men selected because they were known to be healthy have given somewhat different results. The Langley Field group of 204 cases gave a range of differences from 0 to 33. While none of them showed a decreased rate on standing there were 14, or 6.9 per cent, with no change. The greatest increase was only 33 beats and 7 cases, or 3.4 per cent, had an increase of 30 or more beats. The average increase was 12.5 $\pm$ 0.4. In the other physically fit group of 144 men the range of change was from -3 to $+36$. Of these 2 cases, or 1.4 per cent, gave a decrease and 3, or 2.1 per cent, no change when standing; while 3 cases, or 2.1 per cent, showed an increase of 30 or more, the greatest being 36 beats. The average increase was only 13.8 $\pm$ 0.4.

Selected extremes. We have compared other specially selected groups in which the average postural change in the frequency of heart beat was found to be quite different. The comparisons that brought out differences were of men with a rapid or a slow pulse rate; with a high or a low systolic pressure; and men in whom the systolic pressure rose and others in whom it fell when the standing position was taken. One selection included 123 men with a rapid pulse, having in recumbency a rate of 87 or above and in standing of 108 or more. In the group showing a slow pulse rate there were 95 men with a reclining rate of 60 or less and a standing rate of 75 or less. The average increase in pulse frequency for standing over reclining for the rapid pulse group was 22.2 and for the slow pulse group only 12.8. This difference will be discussed later. The high systolic arterial pressure group was composed of 79 cases in which the systolic pressure was 138 mm. or more and the low pressure group of 26 cases in which it was 98 mm. or less. The high systolic group showed an average reclining to standing increase of 14.7 beats and the low pressure group of 20.9 beats. This observation is in harmony with the teaching that the heart is protected against a high arterial pressure by a slowing of its frequency of beating when the pressure rises. However, too much emphasis should not be given to this fact in that the frequency of the pulse for the high systolic group for the reclining posture ranged between 60 and 105 and in the standing posture between 69 and 126; while for the low systolic group the rate in recumbency ranged between 48 and 96 and in the erect position between 72 and 111. Furthermore the number of men with a rate above 100 while standing was 39.2 per cent for the high and only 19.2 per cent for the low systolic pressure group. The normal tempo of pulse for the high is above that of the low systolic pressure group; the average reclining posture pulse frequency for the former was 81.9 and for the

latter 68.3, while the standing was 96.6 and 89.2 respectively. The two groups were more alike during standing. The greater increase in the pulse rate on standing that occurred in the group having a low systolic pressure is quite the opposite from what might be expected when we recall the data on our slow pulse rate group considered above; in the latter a slow recumbency rate gave prospect of a small acceleration during standing.

The influence on the pulse rate of a systolic pressure rise or fall on standing was studied on men whose reclining systolic pressure was within the limits 110 and 122 mm. For the systolic rise group there were used 104 men who, when standing, showed a compensatory rise of 12 mm. or more; and for the systolic fall group 117 men who showed a fall of 8 mm. or more. The average increase for the former was only 13 beats, while for the latter it was 22.8 beats. Some such difference was to be expected since as a rule the heart accelerates more than normally during standing when the systolic pressure falls. For the systolic rise group the pulse rate ranged during reclining from 57 to 105, while standing from 66 to 117; and for the systolic fall groups the reclining rate ranged from 51 to 96, and standing from 69 to 147. In the former there were 15.4 per cent and in the latter 35 per cent with a standing rate above 100. The average recumbent rate was about the same for the two groups, being 75.8 for the former and 74 for the latter; but in standing the rates were 88.8 and 96.8 respectively. The relationship between the postural changes in pulse rate and systolic pressure will be discussed more fully later on.

Predictable postural change. It was pointed out by Guy (19) that the effect of posture varied with the frequency of the pulse, being greater the faster the normal pulse. For rates from 50 to 70 the standing-reclining difference was found to be 9, from 70 to 90 it was 15, from 90 to 110 it was 27, and for 110 to 130 it rose to 39. Graves had earlier shown that if the pulse were but 60 the difference was generally not more than 6 or 8; while if the frequency were raised to 90 or 100 the difference was as much as 20 or 30.

We find that the average amount of change for any chosen range of pulse depends upon whether the reclining or standing rates be taken as the basis for comparison. If the amount of standing-reclining difference be tabulated with regard to the reclining pulse frequency then the predictable change is not the same as when the differences are tabulated with respect to the standing rate. Thus with the reclining pulse as the basis for comparison we find for our group of 2000 cases

that when the rates ranged from 51 to 63 the mean increase on standing was 21 beats, for the rates 66 to 78 it was 18, for between 81 to 90 about 15 and between 93 to 108 only 12. This relation is shown graphically in figure 3, in which the reclining rates are shown as a heavy straight line for reference while the means for standing rate for each group have been plotted in the broken line. The curve of the means has been smoothed by the usual method employed by statisticians (36). For the group in which the reclining rate was 54, the mean standing rate was 76.4, a difference of 22.4; and for the group in which the reclining rate was 96, the mean standing rate was 109.4, a difference of only 13.4. Clearly then when viewed from the reclining posture the more frequent the pulse the less on the average will the heart accelerate its rate when the standing position is taken.

On the other hand when the data on the standing-reclining difference were tabulated with respect to the standing rate it was found that the mean or average decrease was least for those men who had a slow standing rate, and that for those subgroups in which the standing rate was more rapid the reclining fall was proportionately greater. For each increase of 6 beats in the standing pulse rate the standing-reclining difference was correspondingly increased 3 beats. Thus when the standing rate was 60 to 63 the decrease on reclining was 3 beats, for 66 to 69 it was 6, for 72 to 75 it was 9, and so on until at 117 to 120 it was 30. This relationship has been shown graphically in figure 3. In this figure a standing rate of 69 is associated with a mean reclining rate of 62.3, a difference of 6.7; while at the standing rate of 114 the mean reclining rate is 85.5, a difference of 28.5. By comparison on the basis of the observed standing rates, we therefore secure standing-reclining differences somewhat like those reported by Graves (18) and Guy (19). They, however, obtained slightly greater differences.

The opposite results obtained by these two methods of comparison are accounted for by the scattering of cases. We can best get at the facts by examining correlation tabulations. Thus if we take groups tabulated on the basis of the reclining pulse frequency and record the standing rate for each man of the group then the range and the median of the standing rates indicate the distribution within the group. For the group in which the reclining rates were 59–60–61 the range of the standing pulse rate was from 60 to 111 and the median rate 81; for the reclining group 71–72–73 the standing range was from 60 to 120, median 90; for reclining group 83–84–85 the standing rate ranged from 84 to 123, the median 99; and for 95–96–97 the range was from 90 to 129,

median 111. Viewed from the standing rate basis of tabulation the distribution is indicated by the following: for the group with standing rates 71–72–73 the reclining pulse rates ranged from 48 to 78, median 63; for 83–84–85 the reclining range was from 54 to 93, median 69; at 95–96–97 the range from 57 to 96, median 75; at 107–108–109 the range was from 63 to 96, median 84; and at 119–120–121 the range was 72 to 105, median 90. The differences between the group numeral and the median of the range for the two series show an inverse relationship, the differences in one series increasing and in the other decreasing. For the reclining group 59–60–61 the standing median was 81; if therefore the group with a standing rate of 80–81–82 be examined we should expect, if the rate of the reclining pulse gave a dependable indication of what the standing rate is to be, the reclining median to be 60. However, for the 80–81–82 standing group the range in reclining extends from 51 to 81 and the median is 69 instead of 60.

What was found for our large group concerning the amount of reclining-standing difference was also true for our small groups, but because of the smallness of numbers in each there occurred more irregularities which, to some extent, obscured the facts. The Langley Field group of healthy and presumably fit men shows the relationship very well and brings out a difference that exists between men in good condition and the general run of men. The medians of the change for the reclining-standing differences for this group have been compared with those of the 2000 cases in table 1. It will be observed that the law of inverse ratio for the amount of change, as determined for the reclining and standing classes separately, is clearly shown for both; but that the amount of change, as might be expected, is less for the fit group. As viewed from the basis of the reclining pulse the Langley subgroups average from 3 to 9 beats less than the large group. The contrast is most conspicuous for the fit men who have reclining rates between 72 and 87. For the standing basis of comparison the differences are not so great; up to 78 there is an average difference of 3 beats, from 80 to 90 the changes are the same for both groups but above 90 the fit group again averages slightly less.

From the results obtained in our study of the reclining-standing difference in pulse frequency it is evident that a slow pulse in the reclining posture fails to indicate that the increase on standing will be slight, but rather that it is likely to be large, showing a tendency to approach the average or normal standing figure. On the other hand, a slow standing pulse frequency usually means a small retardation of the

heart when the horizontal position is taken. It is further evident that physically fit men show a smaller difference between reclining and standing than do men generally. A slow pulse rate in the reclining and standing positions with a small difference between the two are usually regarded as signs of excellent physical condition.

The correlation between the reclining and standing pulse rates have been determined for 3 of our groups. It was for the 2,000 cases 0.666 $\pm$0.008, for the 200 unselected cases 0.729 $\pm$ 0.022, and for the Langley Field group 0.548 $\pm$ 0.034. While these are high correlations the fact

TABLE 1

The postural pulse rate change shown by the median

PULSE RATE IN RECUMBENCY	INCREASE ON STANDING		PULSE RATE IN STANDING	DECREASE ON RECLINING	
	204 physically fit men	2000 unselected men		204 physically fit men	2000 unselected men
60	18	21	60		3
63	15	21	63	3	3
66	15	18	66	3	6
69	15	18	69	3	6
72	12	18	72	6	9
75	12	18	75	9	9
78	12	18	78	9	12
81	9	15	81	12	12
84	6	15	84	15	15
87	6	15	87	15	15
90		15	90	15	18
93		12	93	15	18
			96	15	21
			99	18	21
			102		21
			105		24

that they are less than unity makes it evident that the pulse frequency during standing is not determined by the reclining rate alone. Other physiological factors help to account for a greater or smaller acceleration in one case than another.

The pulse frequency after physical exercise. It is generally assumed that in vigorous physically fit men the rate of heart does not accelerate as much during a given exercise as in men out of training. Hartwell and Tweedy (20), on comparing athletic and non-athletic women, found that running up and down stairs accelerated the heart rate an average of 10 beats more in the non-athletic women. The rapidity of

the pulse during exercise is chiefly determined by *1*, the speed of movement; *2*, the resistance encountered; *3*, the condition of the individual; *4*, the external conditions under which the work is performed; and *5*, age. Bowen (6), (7) who notes these conditions has also pointed out that the count after exercise is worthless for comparison unless made after exactly the same period of delay in each case and unless the subject is placed in exactly the same position and assumes the same degree of relaxation and repose. Cook and Pembrey (9) lay stress upon the importance of observing the pulse directly after exercise as the rate slows immediately and very rapidly in the trained man. The frequency at the cessation of effort may be taken therefore as a gauge of the degree of distress produced.

Another widely recognized sign of condition is the time required for the pulse rate to return to normal after effort. A quick recovery of the normal rate is characteristic of the physically fit. Bowen (5), (7) has shown that the retardation of the pulse after exercise ordinarily occurs in 3 stages: *1*, a primary rapid decrease; *2*, a plateau of variable duration; and *3*, a secondary slower decrease. Lowsley (29) found after short periods of exertion that the pulse rate usually becomes subnormal, but after fatiguing and exhausting exercises it returns to normal more slowly and only rarely passes into the subnormal stage.

The form of exercise given in our study was that of stepping up on a chair about 18 inches high, 5 times in 15 seconds, as timed by a watch. In order to have the test uniform the subject stood with one foot on the chair at the count one; this foot remained on the chair and was not brought to the floor again until after the count five. At each count he brought the other foot on the chair, and at the count "down" replaced it on the floor. This was timed accurately so that at the end of 15 seconds both feet were on the floor. Immediately, while he stood at ease, the pulse rate was counted for 15 seconds. This form of exercise was chosen because it was something that all men do equally well, it does not require an unusual unfamiliar coördination such as hopping, jumping or the standing run. It had the advantage of uniformity, ease and moderateness which the others do not afford.

Flack and Bowdler (16) conclude from a use of the above form of exercise that an increased rate of over 25 and a return period of over 30 seconds are points calling for careful consideration of the cardiovascular system.

In figure 3, by the use of curves of means, a comparison is given of the pulse rates in standing and immediately after exercise for the 2000 cases.

The curves are most widely separated when the standing pulse is slow. They show a tendency to converge but would meet only when the rates are more rapid than any obtained in this study. The greatest frequency of pulse recorded for standing was 147 and after the exercise 156. In the standing position only 24.6 per cent and after exercise 52.6 per cent had a rate above 100. The mean rate after exercise was 102.42 ± 0.20, the standard deviation 12.97 and the mean increase above the standing rate was 10.43.

In figure 2*B*, the distribution of the 2000 cases with respect to amount of change has been given in a frequency curve. There were 99, or 4.95 per cent, in whom the pulse rate was less immediately after exercise and 110, or 5.5 per cent, who showed no change. The largest increase was 48 beats. There were 0.35 per cent, who gave an increase of 40 or more, and 88, or 4.4 per cent, who gave an increase of 25 or more beats.

The means for the standing rate subdivisions gave an acceleration of 15 beats for those with slow standing rates of 78 or less; 12 beats in groups with rates 81 to 93; 9 for the rates 96 to 111 and only 6 for higher rates. However, the range of exercise increase was large for each group; thus for the standing subdivision 71–72–73 it was between 0 and 42, at 83–84–85 from −9 to +36, at 96 from −18 to +42 and at 108 from −9 to +21.

For the group of 200 men chosen at random in which a greater effort was made to secure an exact standing normal, by having each subject stand at ease 2 minutes before the rate was counted, the mean exercise increase was 12.1 beats and the mean rate immediately after exercise 102.20 ± 0.19. Only 4, or 2 per cent, had a slower pulse rate after exercise and 7, or 3.5 per cent, gave no change. The median acceleration for all the standing rate subdivisions was between 9 and 12 beats. For 3 men whose standing rates were highest, 117, 120 and 126, the exercise changes were 0, −3 and 0 respectively. The largest increase was 36 while only 10, or 5 per cent, had an increase of more than 25.

In the Langley Field group the mean increase was 11.7 beats and the greatest 39. While no one had a decrease in rate immediately at the close of the exercise yet there were 16, or 7.8 per cent, with no change.

The correlation between the standing and exercise pulse rates was 0.789 ± 0.006 for the 2000 cases and 0.801 ± 0.005 for the 200 cases. A summary of the statistical data for the 2000 cases is given in table 2.

Time of return after exercise. Under the conditions of the test, the subject was required to stand at ease for at least 2 minutes after the

exercise. In some men the pulse rate was counted for each 15-second interval, while in others it was only counted at the end of the period of rest. For the 2000 cases the mean rate at the end of the 2 minutes of rest was 86.21 ± 0.21, which was 5.8 beats below the mean standing rate. The tendency for the rate to become subnormal was less common among men with a slow standing rate. This is shown in figure 3 where the mean rate has been plotted for each subdivision and compared with the standing and immediately after exercise curves. The standing curve and the after-rest curve cross at 72; hence for standing rates below 72

TABLE 2

Pulse rate

POSITION	WITH STANDING		M.	P.E.$_m$	P.E.$_o$	σ	FROM STANDING		$\frac{D}{P.E._d}$	Py on x
	r	P.E.$_r$					D.	P.E.$_d$		
Standing...			91.992	0.190	8.472	12.561				
Reclining..	0.666	0.008	74.106	0.155	6.896	10.224	Below 17.886	0.245	73.004	y = 0.819x
Exercise...	0.789	0.006	102.423	0.196	8.749	12.972	Above 10.431	0.272	38.349	y = 0.764x
After 2 minutes..	0.667	0.008	86.205	0.210	9.405	13.944	Below 5.787	0.283	20.449	y = 0.608x

r = coefficient of correlation.
P.E.$_r$ = probable error of coefficient of correlation.
M = mean.
P.E.$_m$ = probable error of the mean.
P.E.$_o$ = probable error of single observation.
σ = standard deviation.
D. = difference.
P.E.$_d$ = probable error of the difference.
Py on x = regression coefficient.

the average was above normal, while for those above 72 the trend was toward subnormal at the end of the 2-minute rest period. The average amount that the pulse rate became subnormal was greatest among men with a high standing rate. From the original tabulations we find a decrease of 3 beats at the end of the rest period for the men whose standing rate was between 76 and 94, while it was 6 beats for from 95 to 109.

It follows from the above that the proportion of men who were slow in returning to normal and subnormal was greatest among those who had a standing rate under 90. There were 489 men whose standing

pulse frequency was above 100; of these 76, or 15.5 per cent, had a rate above normal at the end of 2 minutes; while among 308 men with a standing rate less than 80 there were 122, or 39.6 per cent, still above. Among the entire group of 2000 men 22.6 per cent were still above normal and 63.4 were subnormal at the end of the rest period.

In another group of 217 unselected cases the distribution at the end of 15-second intervals has been recorded. The percentage in which the pulse rate had returned to normal or had become subnormal, was 11.5 at 15 seconds, 15.2 at 30 seconds, 33.8 at 45 seconds, 45 at 60 seconds, 57.4 at 75 seconds and 63.1 at the end of 2 minutes.

In the Langley Field group, selected because they were considered very healthy by clinicians, the return was on the whole more prompt, 93.1 per cent were back to normal or were subnormal in 60 seconds; and only 2 men, 0.98 per cent, were above normal at the end of 2 minutes. In only 16.2 per cent of these men was the return made within 30 seconds, considered by Flack and Bowdler as the outside limit for the physically fit men. If it be concluded that the return is made within 60 seconds by healthy men even then it is evident that there were many in our unselected groups who failed to meet the requirement.

THE ARTERIAL BLOOD PRESSURES. In spite of the fact that blood pressures are nowadays frequently recorded, it cannot be said that the norms of health are clearly established. Blood pressure varies with age, the ingestion of foods and fluids, psychic influence, physical exercise and the time of day. It has been maintained by Dearborn(13) that adequate physical training raises the blood pressure to a definite maximum of efficiency; thus, in trained females the average systolic pressure was 114 mm. and in the untrained 107.9 mm. Middleton (32), on the other hand, observed that as a result of a training period in football there was a decrease in both systolic and diastolic pressures. Dawson (12) found that the effect of training on the resting blood pressure was neither striking nor constant, and Bainbridge (2) concluded that the systolic arterial pressure was not higher during rest in trained than in untrained men.

The normal limits for young adults have been studied by many. Among the more recent observations several may be cited. Dearborn (13), from a series of normal individuals varying in age from 11 to 32, found that the systolic pressures in the brachial artery ranged from 90 to 165 mm., with an average of 116.2 mm. Barach and Marks (3) encountered among 742 male students systolic pressures that ranged from 90 to 200 mm., the mode of their frequency curve was between 120

and 130 mm., while 52.7 per cent of the men had a pressure less than 130 mm. The diastolic pressure ranged from 40 to 130 mm., the mode occurred between 90 and 100 mm. and in only 48.7 per cent of all cases was it under 90 mm. Lee (28) in a study of 662 normal young men obtained systolic pressures that ranged up to 180 mm., with an average 120 mm. In five of the men the diastolic pressure was above 100 mm. and the average for the large group was 80 mm. Alvarez (1) from a study of 2930 men found that the systolic pressure ranged from 90 to 175 mm., the arithmetical mean was 126.5; while 50 per cent of all cases fell between 116.5 and 136.5 mm.

In general it appears to be the opinion that the limits in normal individuals at rest range for systolic pressure from 110 to 135 mm., diastolic pressure 60 to 90 mm., and pulse pressure 30 to 50 mm. The systolic pressure is considered to be an index of cardiac energy expended and indicates the strain to which the arteries are subjected. Taken alone it is of relatively little value. The diastolic pressure is generally considered to be a measure of the peripheral resistance to the circulation and to be an index of vasomotor tone. It indicates the work the heart has to do and is normally considered about three-fourths of the systolic pressure. A pulse pressure persistently as low as 20 or as high as 60 mm. is generally considered pathological. The pulse pressure has been regarded by Dawson and Gorham (11) and others, as representing the volume of the blood wave sent out at each beat of the heart. Ruth Skelton (39) has recently shown, by means of the heart-lung preparation of Starling, that a short sudden contraction of the heart will raise the pressure more and give a bigger pulse pressure than a slow prolonged contraction which expels the same amount of fluid. Accordingly the pulse pressure for a constant volume of output will tend to be higher with a low than with a high arterial resistance. The observations indicate that the pulse pressure is an unsatisfactory index of the amount of blood pumped by the heart.

In spite of all the work that has been done on the subject, there is still no general agreement as to the relative values of the blood pressures in the recumbent and erect postures of the body. In fact, the published data often fail to indicate the posture that was used for the observations recorded. Oliver (33) gave the opinion that recumbency furnishes the standard blood pressure of the individual and the difference observed in the erect posture and recumbency affords evidence of the integrity or failure of vasomotor tone. He finds the systolic pressure about 10 mm. higher in standing than in recumbency. Others do not

agree with this conclusion. Schapiro (37) and Stephens (40) find that the systolic pressure is highest in the reclining position. Cotton, Rapport and Lewis (8) state that the systolic pressure in the arm is recognized as being lower for the standing than for the sitting posture. Karrenstein (25) noted a little lower brachial systolic pressure in the recumbent than in the sitting posture. Erlanger and Hooker (14) found that when the standing posture is assumed from the recumbent or sitting position the systolic and diastolic pressures may either rise or fall, depending upon such circumstances as external temperature and state of digestive organs, and that as a rule the pulse pressure is diminished. Sewall (38) concluded from several hundred observations that in changing from the recumbent to the standing posture there is always a rise in diastolic pressure and a fall in pulse pressure, while in normal cases the systolic pressure does not change materially or shows a rise. According to Hill (23) any influence which weakens the splanchnic vasomotor mechanism interferes with the compensation, with the result that there is a fall in the systolic pressure in the standing posture. Meylan (31) considers systolic pressures for the horizontal posture between 110 and 140, and for the vertical position between 110 and 150 mm. as favorable health signs. Since the effects of passive postural changes, as given by means of a tilting table, give conditions unlike those of muscular effort, the literature of such studies has been omitted here.

Erlanger and Hooker (14) have brought out a further fact regarding the postural changes, namely, that with changes in posture there is an inverse ratio between pulse rate and pulse pressure. Hence when the pulse rate increases, as on standing, then the pulse pressure falls. Because of this there is a tendency for the product of pulse rate by pulse pressure to remain constant. This product is considered an indirect measure of the rate of blood flow and since it is presumably the same in all postures the velocity of the blood flow is probably the same in all postures.

All arterial blood pressures considered in this investigation were determined with the Tycos sphygmomanometer, of which the accuracy of the aneroids was tested from time to time with an ordinary mercury manometer. The auscultatory method of determining the phases of the blood pressure was used, for which a Bowles stethoscope was held in place by means of an arm band placed over the artery just below the compressing cuff. The cuff was invariably placed on the naked upper arm on a level with the heart over the brachial artery and kept

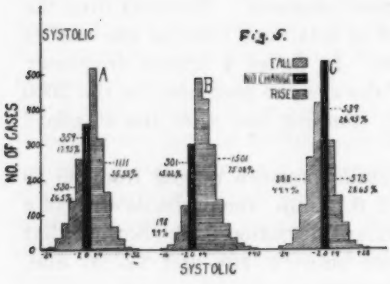
there until all the observations were finished. The systolic pressure was of course read at the appearance of the first sound.

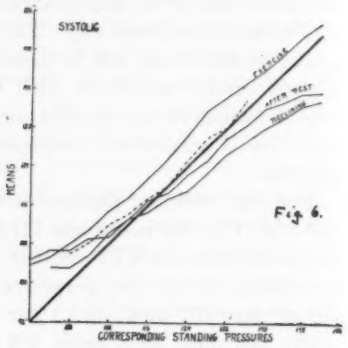
THE SYSTOLIC PRESSURE: *The postural pressures.* The data from the group of 2000 cases will be presented in detail and those of the smaller groups briefly compared with these. In figure 4 appear frequency curves that give the distribution of the systolic pressures for the 2000 cases for 3 conditions, recumbency, standing and after the standard exercise.

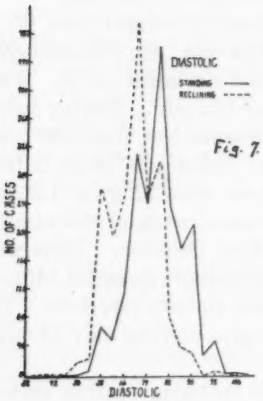
For the reclining posture the systolic pressure ranged from 86 to 154 mm. Hg., the mean was 117.98 ± 0.15 mm., the probable error of a single observation 6.73 and the standard deviation 9.98, showing that two-thirds of all the pressures were between 108 and 127.96 mm. There were 101 men, or 5.1 per cent, with a reclining posture pressure under 100 mm., in 8 cases, 0.4 per cent, the pressure was as high as 150 mm., and in only 28 men, 1.4 per cent, was it above 140 mm.

The systolic pressure for the standing position ranged from 86 to 170 mm., the mean pressure was 120.26 ± 0.18 mm., the probable error of a single observation 7.94 and the standard deviation 11.78. Two-thirds of all the cases fell between 108.5 and 132 mm. During standing 100 men, 5 per cent, had a systolic pressure less than 100 mm., however there were only 45, or 2.25 per cent, below this figure in both the recumbent and standing postures. There were 27 men, 1.35 per cent, in whom the pressure was 150 mm. or more, while of this number in only 3, 0.15 per cent, was it this high in both postures. There were 79 men, 3.95 per cent, in whom the standing pressure exceeded 140 mm. and of these 20, 1 per cent, exceeded 140 mm. in both positions. The man with the highest standing pressure, 170 mm., showed only 130 mm. in the recumbent posture.

In our group of 200 unselected cases the reclining posture systolic pressure ranged from 94 to 154 mm., while the mean was 117.6 ± 0.53 mm.; in the standing position the range was 90 to 158 mm., with the mean 119.16 ± 0.47 mm. So in this group the pressure for the standing posture averaged 1.6 mm. more than in the reclining position. In the large group the standing average exceeded the reclining by 2.3 mm. In the Langley Field group of 204 healthy men the mean pressure for the standing position was 117.72 ± 0.51 mm. and for the recumbent position only 112.46 ± 0.5 mm., the difference between the two postures being 5.3 mm. So here as in the group of 2000 the standing posture pressure exceeds that of the reclining position. The greater standing increase is the only blood pressure feature in which the physically fit







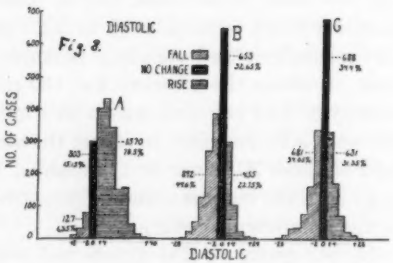


Fig. 5. The change in the systolic pressure. *A*, from reclining to standing; *B*, after exercise when compared with standing; *C*, 2 minutes after exercise, also compared with standing.

Fig. 6. Lines of means of the systolic pressures in which the heavy black line gives the corresponding standing pressures. The broken line gives the standing means when the heavy black line is used to show corresponding reclining pressures.

Fig. 7. The diastolic arterial pressure distribution of 2000 cases.

Fig. 8. The change in diastolic pressure. *A*, from reclining to standing; *B*, after exercise when compared with standing; *C*, 2 minutes after exercise, also compared with standing.

men differ from men generally. In the two small groups only one man had a pressure above 150 mm. in both positions.

The postural change in systolic pressure. Since the difference between the systolic blood pressure in the erect posture and recumbency is usually considered to afford evidence of the integrity or failure of the vasomotor mechanism, in that there should be a rise in the systolic pressure due to the splanchnic compensation which offsets the increased hydrostatic effect of gravity during standing, a study of this subject was undertaken. The amount of change in the systolic pressure when the change is made from recumbency to standing has been plotted for the 2000 cases in a frequency curve in figure 5, *A*. There were 359 men. 17.95 per cent, who showed no postural change; in 1111, 55.55 per cent, the systolic pressure was highest for the erect position; and in 530, 26.5 per cent, it was highest in recumbency. The greatest rise in pressure on changing to the erect posture was 40 mm. and the greatest fall 32 mm. The entire group gave a mean increase of 2.3 mm. on standing. When the amount of change was tabulated by groups with respect to the height of the reclining posture pressures the median change for each group, taken group by group, was an increase of from 2 to 4 mm. However, when the data on change were tabulated with respect to the height of the standing pressures the medians by groups showed that for those men whose standing pressure was under 100 mm. the pressure increased by 8 to 12 mm. when the reclining position was assumed; for the groups with a standing pressure between 100 and 112 the medians indicated no change in recumbency; while above 112 the median pressure showed a decrease in recumbency. Thus the groups with a systolic pressure of 118 to 126 mm. showed a median fall of 4 mm; those of 130 to 138 mm. a median fall of 8 mm., and above that 12 mm. An examination of curves that give the distribution of the cases within each group shows a wide range in the differences; for 175 men in whom the standing systolic pressure was between 106 and 109, the change on reclining ranged from +24 to −8; that is the pressure increased as much as 24 mm. and in others fell as much as 8 mm; the mode of the curve was at +4 and the median at 0. For the 158 men with a systolic pressure of 130 to 133, the range of change was from +12 to −24, the mode of the curve at −4 and the median at −8. These two examples are typical for all groups arranged according to their standing posture. Our smaller group of 200 unselected cases shows the same shifting of the median from that of a rise on reclining for men with a low to a fall for those with a high pressure in the erect position. This tendency of

men with a low standing posture systolic pressure to have a greater pressure during recumbency and of men with a high pressure while standing to have a less pressure in recumbency is also brought out by plotting the line of means of the systolic pressure for the two postures. The lines of the means are shown for the 2000 cases in figure 6. These lines cross at 114 mm. at which point the mean pressure is the same for both postures, while above this the reclining pressure falls and below this it rises. Accordingly for a standing pressure of 108 mm. the mean reclining posture pressure is 109.4 mm.; for a standing pressure of 100 mm. that of reclining is 106.5 mm.; while above 114, when the standing pressure is 120 mm., that of reclining is 117.4; at 132 mm. the reclining is 126.7 mm.; and at 148 mm. the mean reclining is 137.2 mm.

Systolic pressure after a standard exercise. As a rule any exercise which increases the pulse rate will also raise the arterial blood pressure. The more violent the exercise the greater will be the rise in the pressure. Cotton, Rapport and Lewis (8) have shown that within certain limits of effort the rises in blood pressure are controlled by the amount of work done, and the pulse rate more by the rate at which the work is accomplished. Smythe (41) believes that in the majority of cases the systolic pressure is lowered immediately after moderate exercise, and Erlanger and Hooker (14) find very moderate muscular exertion, e.g., walking, may diminish the diastolic pressure. Lowsley (29) from his study of systolic, diastolic and pulse pressure after work found that when these have risen during exertion they return to normal more or less slowly and, continuing to fall, invariably become subnormal and remain in this condition for a considerable period. The return to normal and the time of subnormal pressure is longer the more exhaustive the exertion.

Since it has frequently been shown that the systolic arterial pressure rises very considerably during moderate exercise, it is to be expected that the very easy and simple exercise given in this study would produce some effect. In figure 5, *B*, the amount of change observed in the 2000 cases immediately after exercise has been given in a frequency curve. There were 1501, 75.1 per cent, of the men who showed an increase of from 4 to 36 mm.; 199, 9.9 per cent, a fall of from 4 to 20 mm.; while in 300, 15 per cent, there was no change. The mean increase was 6.72 $\pm$ 0.27 mm. Those with a standing pressure of 106 mm. or less had a median increase of 8 or 10 mm., while those with 130 mm. or more had only 4 mm.

The highest systolic pressure observed after this mild form of exercise was 178 mm., this being reached by a man in whom the standing pressure was 162 mm.; only 9 men, 0.45 per cent, had a pressure above 150 mm. during standing immediately before the exercise; while there were 63, 3.2 per cent, above that immediately after the exercise. There were 2 cases in which the pressure immediately after exercise was only 90 mm. However, while there were 100 men, 5 per cent, with the pressure under 100 mm. when standing at rest, there were only 35, 1.8 per cent, as low immediately after the exercise. The mean pressure after the exercise was 127 ± 0.2 mm. and 120.3 ± 0.02 mm. just before.

The systolic pressure was again determined two minutes after exercising. During the 2 minutes the subject was requested to stand at ease, but not to move. These pressures were compared with the standing normals taken before exercise. The general trend was toward a subnormal pressure which, however, for the entire group averaged only 1.3 mm. below the normal. The amount of change has been plotted so as to show the distribution in figure 5, *C*. At the end of the 2 minutes of rest there were 574, or 28.7 per cent of the cases, still above normal; 887, 44.3 per cent, subnormal; while 539, 27 per cent, were back to normal. For those men whose standing systolic pressure was 116 mm. or less there was a tendency, as indicated by the median, taken group by group, to be back to normal at the end of the rest period; while for those who normally showed a higher pressure than 118 mm., when standing, the median pressure was subnormal, 4 mm. for pressures of 122 to 134 and 8 mm. for those of 138 to 150 mm.

The response of the systolic pressure to exercise and to 2 minutes of rest has been compared with the conditions for standing and reclining in figure 6 where lines of means have been plotted. For the calculation of these lines the cases were first tabulated by dividing the men into groups according to their standing normal systolic pressure, the unit of difference being 4 mm. Thus all cases in which the pressures were 98, 99, 100 and 101 mm. were grouped together under the round number 100; and 102, 103, 104 and 105 grouped under 104. From these curves of the means it is again clear that the usual effect of the exercise is to cause a rise in systolic pressure which is greatest for men with a low and least for those with a high standing pressure. The line of means for 2 minutes after exercise crossing the standing line at about 118, confirms the observations on difference; so again we find that, as a rule, men with a normal standing systolic pressure less than 118 mm. will have a higher pressure 2 minutes after than they had before exercise;

while those having a standing normal above 118 mm. show a decreased pressure after the exercise. The line of means for 2 minutes after exercise occurring as it does between the curves for those of the erect and recumbent postures, suggests the conclusion that as a rule for men with a standing systolic pressure normally above 118 mm. a rest period following the mild grade of exercise such as here employed and the taking of the recumbent posture will both produce a drop in the pressure; while when the standing normal is below 114 mm. or thereabout, both of these conditions will cause a rise in the pressure.

In table 3 will be found a summary of the systolic pressure statistical results obtained from our group of 2000 cases. All comparisons were made with respect to the standing pressure. Many of the data

TABLE 3

Systolic pressure

POSITION	WITH STANDING		M.	P.E.$_m$	P.E.$_o$	σ	FROM STANDING		$\frac{D}{P.E._d}$	Py on x
	r	P.E.$_r$					D.	P.E.$_d$		
Standing...			120.26	0.178	7.943	11.776				
Reclining..	0.740	0.007	117.98	0.150	6.729	9.976	Below 2.28	0.232	9.827	y = 0.874x
Exercise...	0.827	0.005	126.984	0.203	9.092	13.48	Above 6.724	0.270	24.904	y = 0.723x
After 2 minutes..	0.791	0.006	118.920	0.160	7.131	10.572	Below 1.340	0.238	5.630	y = 0.882x

For explanation of symbols see table 2.

summarized in the table have already been discussed. Special attention is called to the coefficients of correlation which are for standing and recumbency 0.740 ± 0.007, for standing and immediately after exercise 0.827 ± 0.005 and after 2 minutes rest following exercise 0.791 ± 0.006. Each correlation is very high. The fact that it is highest for exercise is no doubt due to the fact that exercise quite uniformly causes a rise in systolic pressure while for recumbency and after 2 minutes rest the men fall more or less definitely into two classes of reaction which are determined by the height of the standing normal. The probable errors for single observations and the standard deviations bring out the fact that in the systolic pressure of recumbency there is the least variation and that physiological differences such as those resulting from physical training may express themselves less in recumbency than in the erect posture and exercise.

THE DIASTOLIC PRESSURE. The criterion used in determining the diastolic pressure was the fourth phase of the sound changes and the reading was taken at the lowest point reached by the excursion of the hand on the dial.

The postural pressures. The distribution of the pressures of the 2000 cases during recumbency, standing and immediately after exercise is given by frequency curves in figure 7. The curves show several modes and are, therefore, not as satisfactory as those presented for the pulse rate and systolic pressure. In some degree the method of determining the diastolic pressure may account for the irregularity of the curves in that it is customary to lower the pressure in the cuff by intermittently releasing the air so that pressure falls by steps of 2 to 6 mm. at a time. In this regard the diastolic determination is less satisfactory than the systolic pressure.

Recumbency. The reclining posture diastolic pressure ranged in the 2000 cases from 38 to 106 mm., the mean was 71.63 $\pm$ 0.13 mm., the probable error of a single observation 5.65 and the standard deviation 8.38. It follows from the standard deviation that two-thirds of all cases were between 63.25 and 80.01 mm. A very low diastolic pressure was found in only a few men; in 3 it was below 40 mm.; in fact in only 5 cases was it below 50 mm.; while in 316, 15.8 per cent, it was below 60 mm. In only one man was it above 100 mm. in recumbency; and in 9 cases, 0.45 per cent, above 90 mm.

Standing. In the standing posture the diastolic pressure ranged from 42 to 110 mm., the mean pressure was 79.7 $\pm$ 0.14, the probable error of a single observation 6.12 mm., and the standard deviation 9.07. Two-thirds of all cases fell between 70.63 and 88.77 mm. Only one man had a standing pressure less than 50 mm., while 79, 4 per cent, were under 60 mm. There were 12 cases, 0.6 per cent, with a pressure above 100 mm.

The amount of change. The above comparison of the data for the two postures shows that during standing the diastolic pressure averages 8.1 mm. above that of recumbency. In figure 8, *A*, is a frequency curve showing the amount and direction of change that occurred when the men rose from recumbency to the standing position. The pressure increased as much as 3 to 36 mm. in 1570 cases, 78.5 per cent; fell from 3 to 16 mm. in 127 men, 6.4 per cent; and was not affected in 303, 15.1 per cent.

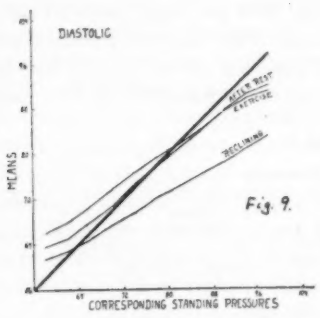
On tabulating the data for the amount of the postural diastolic pressure change with respect to the height of the reclining posture

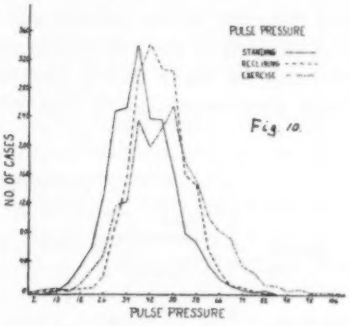
pressure it was found that as a rule those men with a low diastolic pressure during recumbency would show a greater increase in the pressure during standing than men with a high pressure in recumbency. Thus for those whose reclining pressure was between 40 and 66 mm., the median increase for standing was 12 mm.; while for from 74 to 90 mm. the median rise was only 4 mm. When, however, the data for change are tabulated with respect to the height of the diastolic pressure in the erect posture then the predictable change when the reclining position is taken is quite the reverse of that indicated by the recumbency classification. Thus for those in whom the standing diastolic pressure was 60 mm. or less there was on the average no change when the reclining position was taken. For pressures from 64 to 74 the median fall was 4 mm.; from 76 to 82, 8 mm.; from 84 to 90, 12 mm.; and above 90 mm., 12 to 20 mm. There was a considerable amount of scattering of the cases within any subdivision. The following subgroup selections show the degree of this and the relation of the height of pressure to the amount of change. Thus for a group of 67 men, who had standing posture diastolic pressures of 55, 56, 57 and 58 mm., the difference frequency curve showed a range from +12 to −8 with the mode at 0; for a group of 245 whose standing pressures were 71 and 74 mm. the range of difference was from +12 to −20, the mode at −4 mm.; and for a group of 213 cases, whose pressures were 87 to 90 mm., the range was from +8 to −32 and the mode at −12 mm.

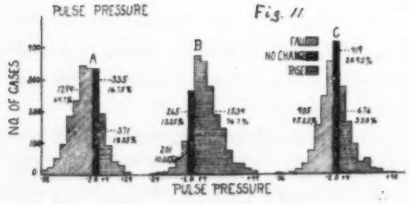
The above relationship of the height of diastolic pressure to the degree of postural change is well shown in the lines of means (see fig. 9) which were established for the entire group of 2000 cases. The line of means for the reclining posture crosses that of the standing at 64. From these it is evident that when the standing diastolic pressure is less than 64 mm., then the pressure in recumbency is likely to be greater than in standing; and when the standing pressure is greater than 64 mm., that in reclining will be less than in standing; and, furthermore, the greater the standing diastolic pressure the greater will be the fall in pressure during recumbency.

Diastolic pressure after the standard exercise. Exercise on the whole caused a slight fall in the pressure. The amount of change noted immediately after exercise has been plotted in a frequency curve in figure 8, *B*. In 892 cases, 44.6 per cent, the pressure when compared with that of standing showed a fall of from 2 to 40 mm.; there were 455 cases, 22.8 per cent, in which it rose 2 to 24 mm.; while 653, 32.6 per cent, showed no change. The mean difference immediately after

exercise was a fall of 2.2 ± 0.2 mm. On determining the median for the standing subdivisions it was found that for diastolic pressures up to 78 mm. this form of exercise, as a rule, does not alter the pressure; for standing pressure 80 to 94 mm., there was a median fall of 4 mm. and above 94 mm. a fall of 8 mm. As was observed under other topics of this study there occurred a considerable scattering of the cases. Thus a frequency curve of changes immediately after exercise for 315







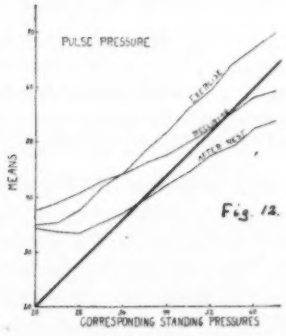


Fig. 9. Lines of means of the diastolic pressure in which the heavy black line gives the corresponding standing pressures.

Fig. 10. The pulse pressure distribution of 2000 cases.

Fig. 11. The change in pulse pressure. *A*, from reclining to standing; *B*, after exercise when compared with standing; *C*, 2 minutes after exercise, also compared with standing.

Fig. 12. Lines of means of the pulse pressure in which the heavy black line gives the corresponding standing pressures.

men, whose standing pressures were 67 to 70 mm., had a range from + 20 to −24 and the mode at 0; and for another subdivision of 213 men, whose standing pressures were 87 to 90 mm., the range was from + 8 to −32 mm., and the median at −4.

The diastolic pressure was again determined two minutes after exercise, the subject standing during the rest period. It was found on comparing these data with the normals for standing (see fig. 8, *C*) that in 681, or 34.1 per cent, the pressure was still below normal; in 631, 31.5 per cent, above normal; and 688, 34.4 per cent, back to normal. The medians of the subdivision showed, after the two minutes' rest, a tendency for those with a standing pressure of 66 mm. or less to be 4 mm. above normal; with pressures between 68 and 82, back to normal;

TABLE 4

Diastolic pressure

POSITION	WITH STANDING		M.	P.E.$_m$	P.E.$_o$	σ	FROM STANDING		$\frac{D}{P.E._d}$	Py on x
	r	P.E.$_r$					D.	P.E.$_d$		
Standing.....			79.70	0.137	6.116	9.068				
Reclining....	0.650	0.009	71.632	0.126	5.652	8.380	Below 8.068	0.187	43.144	y = 0.704x
Exercise.....	0.771	0.006	77.54	0.148	6.602	9.788	Below 2.16	0.202	10.693	y = 0.714x
After 2 minutes.....	0.694	0.008	79.144	0.142	6.351	9.416	Below 0.556	0.197	2.822	y = 0.669x

For explanation of symbols see table 2.

and with pressures of 84 and above, from 4 to 8 mm. below normal. It appears, therefore, that a standing diastolic pressure between 68 and 84 may be considered the physiological normal.

The lines of the means for exercise and after 2 minutes' rest are plotted in figure 9. They present a peculiar situation. The line for immediately after exercise crosses the standing line at 78 mm. and the line for 2 minutes after exercise at about 82 mm. It appears, therefore, that for men with a low standing diastolic pressure, below 78 mm., the effect of moderate exercise will for awhile increase; and for those with a pressure above 80, will somewhat decrease the diastolic pressure.

Correlation data. In table 4 appears a summary of all the diastolic pressure statistical calculations for the 2000 cases, all the comparisons again being made on the basis of the standing pressures. The correla-

tions are again high, that for standing and reclining pressures is 0.650 ± 0.009, for standing and exercise 0.771 ± 0.006, and for standing and after 2 minutes' rest 0.694 ± 0.008. The mean diastolic pressure for each of the 4 conditions of observation indicate that normally the pressure is lowered by recumbency and by a moderate exercise, and that after 2 minutes of rest, it returns to normal. The preliminary standing mean was 79.7 mm., and 2 minutes after exercise was again 79.1 mm. As was true for the pulse rate and systolic arterial pressure so the reclining posture brings all diastolic pressures within the smallest range, as indicated by the standard deviation and the probable error of a single determination. Consequently the least dispersion is to be found during recumbency.

THE PULSE PRESSURE: *Recumbency.* The distributions of the pulse pressures for the 2000 cases during recumbency, standing and immediately after exercise are given in frequency curves in figure 10. In recumbency the pressures ranged from 18 to 86 mm., the mean was 47.41 ± 0.14, the probable error of a single observation 6.4 and the standard deviation 9.5 mm. Two-thirds of all cases fell between 37.9 and 56.9 mm. Since pulse pressures below 30 mm. are regarded as low and above 50 mm. as high it should be noted that 37, or 1.9 per cent, fell below 30 mm. and 438, 21.9 per cent, above 50 mm.

Standing. For the standing position the pulse pressures ranged from 10 to 82 mm., the mean was 41.58 ± 0.17, the probable error of a single observation 7.6 and the standard deviation 11.1. Approximately two-thirds of all cases fell between 30.5 and 52.7 mm. In 256, 12.8 per cent, the standing pulse pressure was under 30 mm.; and in 239, 12 per cent, it was above 50 mm. There were 19 men, 1 per cent, with a pressure under 30 mm. and 170, 8.5 per cent, above 50 mm. in both postures.

The amount of postural change. For the entire group there occurred an average fall of 5.8 mm. in the pulse pressure when the change was made from recumbency to the standing position. The amount of this change has been plotted in figure 11, *A*. There were 1294 men, 64.7 per cent, in whom the pressure fell by from 2 to 40 mm.; 335, 16.8 per cent, who experienced no change; and 371, 18.5 per cent, in whom the pressure increased by from 2 to 22 mm. On classifying the men according to their reclining pulse pressure and the amount of change that occurred with standing, the median amount of change gave a standing posture increase of 4 mm. for reclining pressures of 28 mm. or less, no change when the reclining pressure ranged between 30 and

36 mm., a fall of 4 mm. for pressures between 38 and 44 mm., a fall of 8 mm. for from 46 to 62 mm. and 12 and more when above 64 mm. When the classification was made on the basis of the standing pressure the medians showed that for standing pressures of 22 mm. or less the pressure rose in recumbency 16 mm.; for pressures of 28 and 30 mm., 8 mm.; between 44 and 54 mm., no change; and for from 56 to 68 mm. a fall of 4 mm. in recumbency. It appears that where the reclining pulse pressure is low there will, as a rule, occur either a slight rise or no change when the erect posture is assumed; while for the majority of those in whom the standing posture pressure is low, 30 mm. or less, the pressure will show a decided rise in recumbency. The lines of the means given in figure 12 bring out the above relationship in graphic form. The lines of means have been smoothed by the usual method but even so they conform to the changes stated above. The reclining line crosses that for the standing posture at 56 mm. For men with a pulse pressure of 56 mm. or above during standing, recumbency caused a drop; and for those with less than 56 mm., recumbency caused a rise in this pressure.

The influence of the standard exercise on pulse pressure. The amount of change immediately after exercise and again 2 minutes later is given in frequency curves in figure 11, *B*. The mean pulse pressure was 50.4 ± 0.2 mm. immediately after exercise and 40.8 ± 0.2 after 2 minutes of rest. The exercise caused a rise of from 2 to 40 mm. in 1534, 76.7 per cent; no change in 265, 13.3 per cent; and a fall of from 2 to 24 in 201, 10 per cent, of the cases. The average increase and also the median for all subgroups was 8.8 mm. when the men were classified according to their standing pressure.

Two minutes after the exercise the mean pulse pressure for the 2000 cases was 40.8 mm., which was only 0.8 mm. below the normal standing mean. However, in spite of this apparent similarity, there occurred a great amount of change among the men with respect to the two pressures. In figure 11, *C*, the changes have been plotted and show that the pressure in 676, 33.8 per cent, was to from 2 to 32 mm. above normal; in 419, 21 per cent, it had returned to normal; while in 905, 45.2 per cent, it had become subnormal by from 2 to 36 mm. When the changes were recorded by grouping the men according to the standing pressures, the medians showed that, for all groups in which the standing pulse pressure was 28 mm. or less, the pressure at the end of 2 minutes was still above normal by from 4 to 12 mm. For groups with pressures from 30 to 42 mm. the median was at 0; and for groups with

the pressures above 44 the majority showed a subnormal pressure. The rest after exercise, therefore, tends to bring the individuals more compactly together so that there is less dispersion within the group.

The lines of means following exercise and after 2 minutes of rest are given in figure 12. The almost constant effect of exercise is an increase in pulse pressure, the line of means for the pressure after exercise is constantly above the line for the standing posture pressure by from 6 to 15 mm. However after 2 minutes of rest the usual result is for those with a standing normal above 39 mm. to have had a sufficient fall to bring the pulse pressure below normal. Since the lines cross at 39 mm. it appears that if the standing pulse pressure be below 39 mm.

TABLE 5

Pulse pressure

POSITION	WITH STANDING		M.	P.E.$_m$	P.E.$_o$	σ	FROM STANDING		$\frac{D}{P.E._d}$	Py on x
	r	P.E.$_r$					D.	P.E.$_d$		
Standing....			41.584	0.167	7.457	11.056				
Reclining...	0.578	0.010	47.412	0.143	6.410	9.504	Above 5.828	0.219	26.612	y = 0.673x
Exercise....	0.700	0.008	50.40	0.214	9.556	14.168	Above 8.816	0.272	32.411	y = 0.897x
After 2 minutes.....	0.661	0.008	40.816	0.155	6.927	10.272	Below 0.768	0.228	3.368	y = 0.711x

For explanation of symbols see table 2.

that the standard exercise rather permanently increases the pulse pressure.

A summary of the statistical relationship of the pulse pressure for the postures and exercise is given in table 5. The coefficients of correlation are all high, indicating that the changes of posture, of exercise and of rest after exercise occur in the same direction and in much the same measure in the vast majority of men.

Pulse pressure $\times$ *pulse rate.* The product of the pulse pressure by pulse rate has been widely used by physiologists as an index of the per-minute volume of the heart output. As a result of Skelton's (39) observation on pulse pressure this now, however, appears to be of doubtful value. We have, however, summarized a study of this product for our 2000 cases. In table 6 we have ventured to present these because it is probable that the large number of cases included will

tend to obscure the irregularities noted by Skelton, and we have also given the distribution of cases in recumbency, standing and exercise in the curves of figure 13. Erlanger and Hooker (14) showed, from a study of two men, that there was a tendency for the product of pulse rate by pulse pressure to remain constant in the several postures of the body. Our means for the reclining and standing positions were 3461 and 3735 respectively. Thus according to the former interpretation there occurred a small increase in the per-minute output of the heart during standing. This would be the case if the output of the heart per minute runs parallel with the consumption of oxygen. It should be noted that at the end of 2 minutes of rest following the

TABLE 6

Pulse pressure × pulse rate

POSITION	WITH STANDING		M.	P.E.$_m$	P.E.$_o$	σ	FROM STANDING		$\frac{D}{P.E._d}$	Py on x
	r	P.E.$_r$					D.	P.E.$_d$		
Standing...			3735.0	16.57	740.601	1098.0				
Reclining...	0.663	0.008	3461.0	13.84	618.854	917.5	Below 274.0	21.589	12.691	y = 0.794x
Exercise...	0.666	0.008	5045.0	23.71	1059.302	1570.5	Above 1310.0	28.926	45.288	y = 0.466x
After 2 minutes..	0.658	0.008	3415.5	14.96	671.127	995.0	Below 319.5	22.324	14.312	y = 0.727x

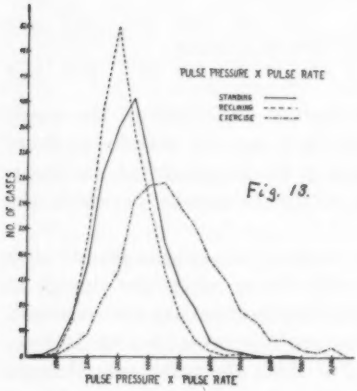
For explanation of symbols see table 2.

standard exercise the product indicated an output by the heart about equal to that found for recumbency.

POSTURAL CORRELATIONS. In order to ascertain whether the several postural circulatory changes are dependent upon one another, and, if so, to what degree, the amount of change for each factor has been correlated with the changes of each of the other factors. The usual procedure of recording the data has been followed and from these tabulations the coefficient of correlation and other relationships have been established.

Pulse rate and systolic pressure. It has frequently been pointed out that when the pressure in the arteries falls the pulse rate increases, and when it rises the pulse rate decreases; or, in other words, there is a

certain inverse relationship between pressure and pulse rate. Marey taught that the two varied inversely to one another. If in the individual the frequency of the heart beat varies with the systolic pressure, whereby a decrease in systolic pressure is associated with an increase in the pulse rate and vice versa, then in a group of men taken collectively this relationship should also be evident. Since Crampton (10) in his "blood ptosis" physical efficiency test, has prepared his rating table on the assumption that when the change is made from recumbency to standing a fall of 1 mm. in systolic pressure is associated with an increase of 2 heart beats; it seemed that it might be useful to evaluate these changes



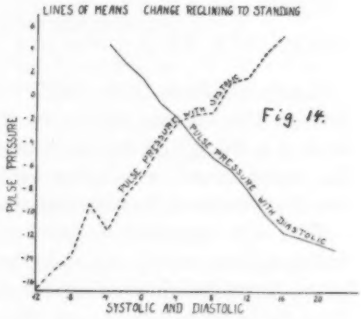


Fig. 13. The distribution of 2000 cases.

Fig. 14. Lines of means showing the postural changes in systolic and diastolic pressure and the corresponding changes in pulse pressure.

statistically. The coefficient of correlation for the recumbent to standing posture changes of systolic pressure and the pulse frequency was for the 2000 cases only -0.213 ± 0.014, for the 200 unselected cases -0.135 ± 0.47, for the Langley Field 203 healthy cases -0.039 ± 0.047. The last two groups appear to have been too small to bring out the relationship which actually does exist between the two sets of changes. However, even for the 2000 cases the correlation is decidedly low, thus indicating that the postural changes in systolic pressure and pulse rate are not regularly and constantly interdependent and influenced by a common factor. It is well to recall that even though Wickwire (45) found that Marey's law of inverse ratio of rate and

pressure holds under many conditions, yet she pointed out that if conditions should change in such a way as to demand a high systolic pressure and a more rapid heart rate then the two controls might be dissociated.

Another way of getting at the systolic pressure pulse rate relationship is to determine the mean amount of pulse rate change for each subgroup when the cases are classified according to the amount of postural systolic pressure change. The following are the results of such a treatment of the 2000 cases:

Postural change from recumbency to standing

Systolic pressure in millimeters												
−12	−10	−8	−6	−4	−2	0	+2	+4	+6	+8	+10	+12	+14
Corresponding pulse rate increase in beats												
24.9	22.5	21.6	19.8	19.8	18.8	19.2	17.9	17.2	17.0	15.9	15.9	14.3	13.4

It appears from these data, which include only 1848 of the cases, that on the average for every change of 1 mm. in systolic pressure there is a change of only 0.44 of a beat in the frequency of the heart. The mean heart rate being highest in the subgroups in which the systolic pressure falls with standing.

That it is impossible to predict, for a given person who stands after reclining, how much the pulse rate will change when the change in systolic pressure is known is evident when distribution data are examined. Thus there were 119 men in whom the systolic pressure rose 10 mm. when taking the standing position. For them the pulse rate changes ranged from 0 to 36, with a mean acceleration of 15.9 beats. There were 51 men in whom the systolic pressure fell 10 mm. when standing and in them the pulse rate changes ranged from −3 to + 39 beats. The ranges of the changes in pulse rate for these two groups were almost identical. For 45 men, who gave no postural change in pulse rate, the systolic pressure changes on standing ranged from a rise of 14 mm. to a fall of 14 mm.; the mean change was a rise of 3.6 mm. For 258 of the men, who had a standing increase of 12 beats in the pulse rate, the systolic pressure changes ranged from +24 to −18 mm. These distributions are typical for all subdivisions and make it clear why the coefficient of correlation for these changes is so low. According to these findings it appears that it has been customary to place too much emphasis on the interdependence of the pulse rate and systolic pressure postural changes.

Pulse rate and diastolic pressure. The coefficients of correlation for the reclining-standing postural changes of pulse rate and diastolic pressure was only 0.117 ± 0.015, which is ordinarily considered to be a negligible correlation. From a determination of the mean pulse rate change for the subgroups arranged according to the diastolic pressure postural change it appeared that for every increment of a change difference of 4 mm. in the diastolic pressure there was an average acceleration of one beat in pulse frequency. The curve of the means, however, was not smooth. It appears, therefore, on the whole that the relationship between the pulse rate and diastolic pressure is less definite than that between pulse rate and the systolic pressure.

Pulse rate and pulse pressure. Erlanger and Hooker (14), from determinations of the pulse rate and pulse pressure on two men, made the deduction that changes in these show an inverse ratio to each other. For our 2000 cases the coefficient of correlation for the postural changes of these was -0.290 ± 0.014. While the correlation is rather low it does show, nevertheless, the inverse relationship. On determining the line of the means of the pulse rate change and plotting it with respect to the pulse pressure change, it became evident that the greater the fall in pulse pressure, when the standing posture is taken, the larger on the average is the acceleration in the pulse rate. The change, as shown by the means for the subgroups, was approximately 3 mm. for pulse pressure to one beat in pulse frequency. For the subgroup in which on standing there was a rise of 10 mm. in the pulse pressure, the pulse rate showed a mean increase of 11.6 beats; for the subgroups that showed no postural change in pulse pressure, the mean increase in pulse rate was 15.7 mm.; when there was on standing a fall of 10 mm. in pulse pressure, the mean pulse rate increased 18.2 beats; and when the pulse pressure fall was 20 mm., the pulse rate increased 22.2 beats. The differences between these subgroup differences were for pulse pressure 30 mm. and for pulse rate 10.6 beats which is approximately the 3 to 1 ratio.

Pulse pressure and systolic pressure. The relationship between the postural changes of systolic and pulse pressure is of necessity very close. The coefficient of correlation for these two sets of changes was $+ 0.642 \pm 0.009$. The plotted line of the means for the pulse pressure postural changes determined with respect to the degree of systolic pressure change gives approximately a straight line. From this (see fig. 14) it is evident that when the systolic pressure shows a rise of 10 mm. there is, as a rule, little or no change in the pulse pressure. If

the rise in systolic pressure is more than 10 mm., then the pulse pressure will increase; but when the rise is less than 10 mm. or shows a fall then the pulse pressure will decrease proportionately to systolic compensatory failure. Our data show the mean changes in pulse pressure to be as follows: with a systolic rise of 16 mm. the pulse pressure rose 5.1 mm; with a systolic rise of 10 mm. pulse pressure rose 1.1 mm.; with no change in systolic the pulse pressure fell 6.9 mm., approximately the average rise in diastolic pressure; and with a systolic fall of 10 mm. the pulse pressure decreased 15 mm. Therefore, for every change of 5 mm. in the systolic pressure there occurred approximately a 4 mm. change in the pulse pressure. A standing posture systolic pressure compensatory rise of 10 mm. has often been considered the normal response of the healthy individual. Our results indicate that a rise of 10 mm. is normal because this means, on the average, a maintained level in the pulse pressure. Insofar as the systolic pressure fails in reaching the normal compensatory rise then by a ratio of approximately 5 to 4 will the pulse pressure fall.

Pulse pressure and diastolic pressure. It has been shown by the use of curves of means that, as a rule, when the diastolic pressure in recumbency is above 64 mm. the pressure on standing will show a compensatory rise. A standing posture rise in diastolic pressure usually is associated with a fall in the pulse pressure. The coefficient of correlation for the postural changes in pulse and diastolic pressures for the 2000 cases was -0.592 ± 0.01. This is somewhat less than that for pulse and systolic pressure and indicates that the pulse pressure changes are more intimately associated with those of systolic than those of diastolic pressure. The signs of the two correlations show that the pulse pressure varies directly with the systolic and inversely with the diastolic pressure change.

In accordance with the plan used thus far we have established the curve of means for the pulse pressure changes and have plotted this in figure 14 with respect to the diastolic pressure change. The data show that when there is no change in the diastolic pressure on standing there is a mean increase of 0.6 mm.; in pulse pressure with a rise of 10 mm. there is a drop of 6.1 mm.; and with a rise of 20 mm., a fall of 12.7 mm. in the mean pulse pressure. Roughly, therefore, the ratio of these changes is 3 to 2; or for every change of 3 mm. in the diastolic pressure there occurs an opposite change of 2 mm. in the pulse pressure.

Systolic and diastolic pressure changes. That the interplay and dependence of these two factors upon each other is not close is shown

by the coefficient of correlation which was only 0.219 ± 0.014 for the 2000 cases.

A curve of the means for diastolic postural change when plotted against the systolic pressure postural change brings out a relationship in which a systolic fall is associated with a smaller than ordinary diastolic pressure rise, and a systolic compensatory rise with a greater diastolic pressure rise. The relationship was roughly 4 to 1, in which a change of 4 mm. in the postural systolic pressure was associated with 1 mm. in the diastolic pressure.

It is evident from the above correlations that some degree of causal relationship does exist between certain of the circulatory factors considered. However, in every relationship the size of the coefficient indicates that no two of the factors are so intimately related that one of them stands as the only or sole cause of the changes in the other. In each relationship it appears that there are other conditions and circumstances that modify the degree of action. The natural conclusion is that a judgment regarding the efficiency of the cardiovascular mechanism and physical fitness can not be made by noting the postural reactions of a single factor. The changes in pulse rate seem to be determined in large part by factors outside the domain of this investigation. They vary in some slight measure inversely with the systolic pressure changes and indirectly, through the relationship of the systolic pressure to pulse pressure, they also vary inversely with pulse pressure. There is almost no connection indicated between pulse rate and the diastolic pressure. Postural variations in the height of the systolic pressure affect in only a slight degree the diastolic pressure. The variations of each of these also must be accounted for by other factors outside the field of these observations. The high correlations between pulse pressure and the systolic and diastolic pressure is no more than was to be expected. The point to be noted here is that in postural changes the pulse pressure varies directly with the systolic and inversely with the diastolic pressure.

The coefficients of correlation for exercise changes. The standard exercise of stepping up on a chair 5 times in 15 seconds was used and the correlations are for the changes as determined by a comparison with the standing posture normals. Certain of the factors showed a well-defined relationship, but for some of them not the slightest connection was indicated.

Pulse rate and systolic pressure. The coefficient of correlation was only 0.078 ± 0.015 and apparently indicates that the changes in

systolic pressure resulting from this form of exercise are in no degree determined by the change in pulse rate. A study of the line of means likewise failed to establish an exercise relationship between these two factors.

Pulse rate and diastolic pressure. The coefficient of correlation was -0.245 ± 0.016. No relationship between these factors was brought out by the lines of means.

Pulse rate and pulse pressure. The coefficient of correlation was only 0.102 ± 0.002.

Pulse pressure and systolic pressure. As was to be expected, there was a high coefficient of correlation for these exercise changes, being 0.773 ± 0.006. In a large majority of men the exercise caused an increase in the systolic pressure and parallel with this increase there occurred a rise in the pulse pressure.

Pulse pressure and diastolic pressure. The coefficient of correlation was -0.980 ± 0.014, the highest observed in our study. Apparently, the pulse pressure changes of exercise follow the diastolic somewhat more closely than they do the systolic pressure changes.

Systolic and diastolic pressure changes gave a coefficient of correlation of only -0.041 ± 0.015.

It is evident that the exercise changes in pulse rate, systolic and diastolic pressure are not dependent upon each other and, in so far as they give evidence of physical efficiency, must be considered separately.

A COMPARISON OF THE CIRCULATORY FACTORS AND CHANGES IN GROUPS SELECTED BECAUSE OF OPPOSITE CONDITIONS. The selections contrast men with slow and rapid pulse rates; high and low systolic arterial pressures; high and low diastolic pressures; high and low pulse pressures; men in whom the systolic pressure rose with others in whom it fell during standing; men in whom the diastolic pressure rose with others in whom it fell during standing; and men in whom the pulse rate did not change with others in whom it retarded during standing. In figure 15 the mean condition of the several circulatory factors for each group is given in curves that bring out the changes in recumbency, standing, after the standard exercise and after 2 minutes' rest after exercise. With these curves have also been plotted the general types of reaction which were shown by the larger group of 2000 cases.

Generally, on changing the posture from recumbency to standing the pulse rate accelerates moderately, the systolic pressure rises several millimeters, the diastolic rises somewhat more than the systolic pressure, while the pulse pressure slightly decreases. Immediately after the

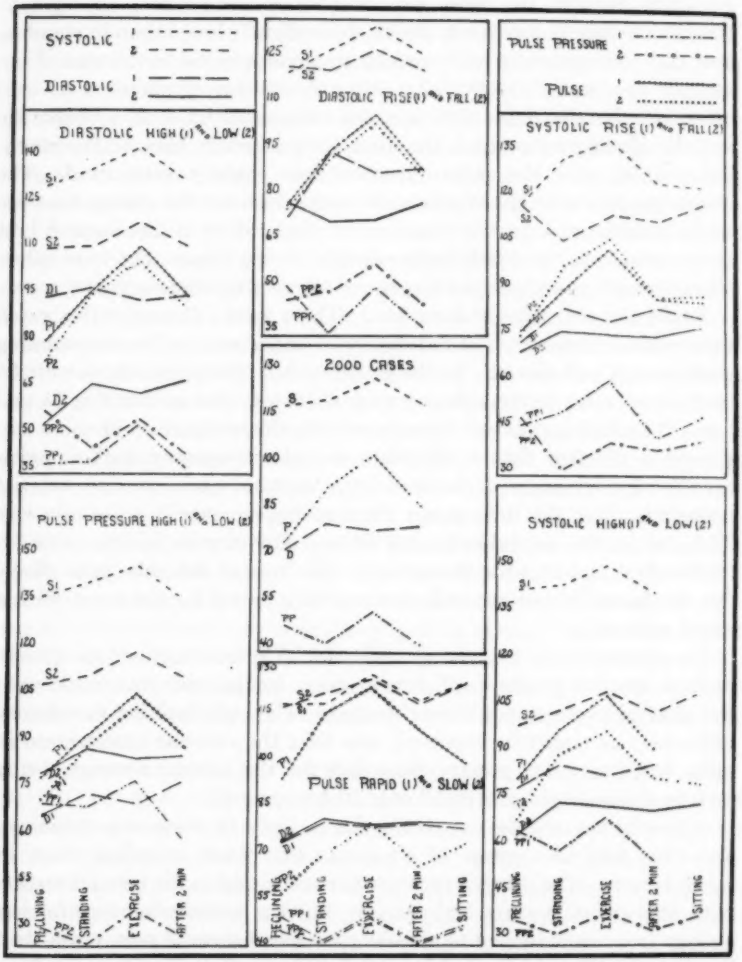


Fig. 15. Comparisons of the pulse rate and arterial pressures in reclining and standing postures, immediately and 2 minutes after exercise.

standard exercise the pulse rate is further accelerated, the systolic pressure definitely increased, the diastolic slightly lower than in standing and the pulse pressure increased by an amount equal to the sum of the systolic rise and diastolic fall. After 2 minutes of standing at ease after exercise the pulse rate becomes subnormal by 4 or 5 beats, the systolic slightly subnormal, the diastolic practically back to the standing normal, and the pulse pressure very slightly subnormal. The above changes give the standard of comparison for the groups that are to be contrasted. In the comparisons that follow it has seemed best to contrast only the conditions as shown by the means that were calculated for each group and not to present the distribution curves.

Rapid and slow rates of heart beat. There were 123 men with a rapid pulse whose rates were in recumbency 87 and above and in the standing position 108 and above. In the group with a slow pulse, there were 95 men whose rates in recumbency were 60 or less and in standing 75 and less. The striking feature brought out by the comparison of these two groups is the fact that a difference in pulse frequency does not give occasion for an unusual change in the postural and exercise arterial pressures. For the first group the standing increase in pulse rate was 22.1 and for the second only 10.8 beats. The exercise increase was 7.6 for the first and 10.5 for the second. The sum of the pulse rate effects due to change in posture and exercise was greatest for the group with a rapid pulse rate.

No postural pulse rate change. In the 35 cases examined no clearly defined arterial pressure difference, when comparison was made with the general average, could be detected. The only striking circulatory difference, as might be expected, was that the exercise acceleration in pulse frequency was greater than that for the general average, being 18.6 for this selected group and only 10.4 in general.

A retardation in pulse rate on standing. Only 14 cases were examined and they had an average of 6.8 beats less when standing than in recumbency. The arterial pressure factors varied in the usual direction with changes of posture and exercise, but the systolic increase for the change from recumbency to standing averaged about 5 mm. more than is generally the case, and this tended to keep the pulse pressure from falling. The greater rise in systolic pressure is in harmony with the coefficient of correlation established for the systolic pressure and pulse rate changes in the previous section of our paper. However, 3 out of 14 of the men showed a fall in the standing systolic pressure of 2 mm. which indicates that a retardation in pulse rate is not necessarily compensated

for by a proportionate rise in the systolic pressure. The exercise pulse rate increase was above the average, being 21.6 instead of 10.4.

High and low arterial systolic pressures. The selections were made on the basis of the pressure in recumbency and from these 79 cases with a pressure of 135 mm. or more and 26 cases with 98 mm. or less were chosen. The postural means as determined for these 2 groups differed from the general average in every circulatory factor. The high systolic pressure group had a mean pulse rate above the general average in the 2 postures and after exercise. As usual the pulse accelerated from recumbency to standing and with exercise, but the postural change increase was less while the exercise increase was greater than usual, so that the sum of the two about equalled the general average. For the low systolic group the mean pulse rate was below the general average, but the pulse responded to changes in posture and exercise in the usual degree. The means for the diastolic and pulse pressures were above the general average in the high systolic and below in the low systolic pressure group. The postural and exercise changes in the diastolic and pulse pressures were in the usual direction, but the changes were greatest among the men with a high systolic pressure. The inverse relations between pulse rate and the systolic pressure and pulse pressure shown by the study of coefficients of correlation are not in evidence when these two groups are contrasted. It appears, therefore, that the circulatory mechanisms of these men were tuned, so to speak, on different levels. Or it may be that the high pressure group included men nervously wrought up. Observations made by the psychologist as these men began the rebreathing experiment do not support the suggestion of nervousness.

A comparison of groups in which the systolic pressure rose or fell on standing. There were used 104 cases in which the systolic pressure showed on standing a compensatory rise of 12 mm. or more, and 117 who showed a fall of 8 mm. or more. In conformity with the relationship already discussed, the pulse rate in the change from recumbency to standing accelerated slightly less, 4 beats, than the general average in the group that experienced the standing posture systolic pressure rise; and accelerated somewhat more, about 5 beats, than the average in the group in which the systolic pressure fell on standing. The exercise increase was the same as that of the general average in both these groups. The systolic pressure increase following exercise was for both about the same as that of the general average. While the diastolic pressures were not greatly altered, yet in the group that had the systolic pressure

rise during standing, the diastolic pressure changes of posture and exercise were slightly above the general average. The pulse pressure changes in these two groups of course reflected the systolic pressure changes. Consequently, the pulse pressure during standing was increased in the group with a rise in systolic pressure and much decreased in the group with a fall in the systolic pressure. The usual exercise pulse pressure increase was present in both groups.

High and low diastolic pressures. There were examined 37 cases in which the diastolic pressure in recumbency was 90 mm. or above, and 50 cases in which it was between 38 and 56 mm. The means as established showed that the pulse rates were quite like those of the general average. The systolic pressures for the high diastolic were 12 to 15 mm. above and for the low diastolic group 8 to 10 mm. below the general average. While the postural and exercise systolic and diastolic pressures changed in the usual directions, yet for the high diastolic group the changes in systolic were greater and in the diastolic less than the general average or than those of the low diastolic group. The pulse pressure naturally reflects the systolic and diastolic pressure changes and so, during standing, was slightly higher in the high and a little lower in the low diastolic pressure group than the general average. This was not, however, affected nearly as much by the diastolic selections as by the systolic high and low selections.

Changes associated with a rise or fall in the diastolic pressure when the standing position is taken. There were examined 83 cases in which the diastolic pressure rose 20 mm. or more and 75 cases in which it fell 4 to 16 mm. These differences were not reflected in the pulse rate changes. The systolic pressure rise on standing was distinctly greater than usual for the diastolic rise group and less than ordinary for the diastolic fall group.

The exercise changes were of the usual magnitude for both groups. The postural change in pulse pressure gave the usual standing fall in the diastolic rise group and a slight rise in the diastolic fall group. There was always a tendency for the systolic pressure to compensate for changes in diastolic pressure, so that the pulse pressure was not greatly altered.

High and low pulse pressures. The selections included 42 cases in which the pulse pressure during reclining was 62 mm. or above and 59 in which it was 28 mm. or less. In this selection men with high and low systolic pressures are again brought into contrast and the differences are quite like those already described.

From the contrasting of these extreme conditions the impression is gained that the pulse rate and the arterial pressure factors may appear together in all sorts of combinations and that there is no causal relationship between them. A study of the individual cases within the groups even more decidedly gives the feeling of indiscriminate or chance association of all the factors in men who are apparently in the best of health. The comparisons, however, confirm on the whole the relationships established in the study of the coefficients of correlation. It appears from the comparisons that the postural rise or fall in the systolic pressure will, as a rule, influence the pulse rate change in some slight degree; that a postural rise or fall in the diastolic pressure will be compensated for by a sufficient change in the systolic pressure to maintain the pulse pressure within the general range of change; that the postural pulse pressure is altered more by changes in the systolic than in the diastolic pressure; and that a high or a low systolic pressure is ordinarily associated with a high or low tuning of the entire cardiovascular mechanism.

SUMMARY

A statistical study has been made of the pulse rate and the three arterial pressures, systolic, diastolic and pulse pressure under four conditions, reclining, standing, immediately after a standard exercise and 2 minutes after the exercise. Four main groups of cases were used, supplemented in certain instances by other specially selected groups. Two groups are composed of unselected cases of aviators, while two other groups are composed of aviators specially selected by clinicians as physically fit.

Pulse rate. In the unselected groups a reclining pulse rate of 74 and 75 rose to 92 and 90 on standing, to 102 after exercise, and after 2 minutes was slightly below the original standing rate in the majority of cases. In the selected groups of physically fit men a reclining pulse rate of 72 and 70 rose to 86 and 83 on standing and to 97 and 95 after exercise. The superiority of the fit groups seemed to be most clearly demonstrated by the lower standing rate, a smaller reclining to standing increase, and a more rapid return to the normal standing rate after exercise.

Taking the reclining pulse rate as a basis, it was found that the reclining to standing pulse difference varied inversely with the reclining pulse rate, i.e., the higher the reclining pulse the less the increase and vice versa. On the other hand it was found that if the standing pulse

rate were taken as a basis, the reclining to standing difference varied directly with the standing pulse, being less with a low standing pulse and greater the higher the pulse.

Systolic pressure. In the large group of 2000 unselected cases, the mean reclining systolic was 118 and the standing systolic 120, indicating an increase on standing. The systolic of a physically fit group rose from a reclining figure of 112 to 118, a greater increase than in either of the other groups. It was noted that those cases having a low standing systolic had an increase on reclining, the high standing systolic had as a rule a decrease, while between these was a group which tended to remain the same. The systolic uniformly tended to rise after the exercise, the lower standing pressures tending to a greater increase. Two minutes after the exercise nearly half the cases showed a slightly subnormal pressure. The higher the original standing pressure, the more marked was this tendency.

Diastolic pressure. A reclining diastolic of 72 rising to 80 on standing, falling 2 mm. after exercise and returning to the original standing level in 2 minutes is the average picture. A low diastolic will tend to rise more on standing and a high pressure will rise less. Also the fall after exercise does not usually occur in cases of low standing pressure, but the higher the pressure the greater the tendency to fall after exercise.

Pulse pressure. A reclining pulse pressure of 47, falling to 42 on standing, increasing to 50 after exercise and falling 2 mm. below the original standing after 2 minutes represents the mean. The higher the reclining pulse pressure the greater the tendency to fall on standing, while a low pulse pressure might remain the same or even rise. While exercise increases the pulse pressure quite uniformly, the pulse pressure 2 minutes later is subnormal in those cases with a high standing pulse pressure but in those with a low pressure the tendency is to remain slightly above normal.

Postural correlations. The relations of various circulatory changes have been tested by correlations which show in some cases slight and in other cases no interdependence. The correlations of reclining to standing changes indicate a slight inverse ratio between pulse rate and systolic pressure changes, the interdependence of these two factors apparently having been unduly emphasized. There is also a slight inverse relationship between pulse rate and pulse pressure. The correlations for exercise changes show a high correlation between systolic and pulse pressures and an even higher correlation between diastolic and pulse pressures, all other exercise correlations being negligible.

Selected groups. A study of groups representing the extremes of pulse rate, systolic, diastolic and pulse pressures and also groups in which one of the pressures rose on standing and other groups in which the same pressure fell seems to show that the relationships of pulse rate and arterial pressure are little influenced by these extremes. It appears from the comparisons that the postural rise or fall in the systolic pressure will, as a rule, influence the pulse rate change in some slight degree; that a postural rise or fall in the diastolic pressure will be compensated for by a sufficient change in the systolic pressure to maintain the pulse pressure within the general range of change; that the postural pulse pressure is altered more by changes in the systolic than in the diastolic pressure; and that a high or a low systolic pressure is ordinarily associated with a high or low tuning of the entire cardio-vascular mechanism.

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STUDIES IN FATIGUE

XI. The Effect of Intravenous Injection of Massive Doses of Adrenalin upon Skeletal Muscle at Rest and Undergoing Fatigue

CHARLES M. GRUBER

From the Department of Pharmacology, Washington University School of Medicine, St. Louis

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The accumulated data on the action of adrenalin upon skeletal muscle, since the publication of the first work, can be divided into two groups: those in which the height of muscular contraction was increased and fatigue alleviated, and those in which muscular activity was depressed. A number of questions have not been sufficiently considered in the discussions, e.g.: *a*, Does adrenalin act the same upon non-fatigued muscle as it does upon fatigued muscle? *b*. What effects have massive doses of adrenalin upon the height of muscular contraction of both non-fatigued and fatigued muscle when injected intravenously? *c*. What effect, if any, has the rate of injection upon the results from adrenalin? *d*. Does adrenalin neutralize or destroy the so-called fatigue products? *e*. What effects have alterations in the rate of stimulation, in tension or in weight to be lifted upon the results? *f*. Is the action the same upon excised perfused muscle as it is upon the intact muscle? *g*. Where is the point of action of adrenalin?

In view of these unsolved questions the author has set out to determine their solutions if possible, and to explain why some investigators have reported depression of muscular activity and others the opposite action.

Inasmuch as a complete review of the literature on the action of adrenalin upon skeletal muscle has been given before by the author (1), we shall merely report in detail here those researches which have been published since the previous communications.

Working upon frogs and toads, Guglielmetti (2) found that after complete fatigue of the voluntary muscles, adrenalin[1] (1 ml. of a 1:1000

[1] In giving the review the author will adhere to the terminology used by the original writers.

solution) injected subcutaneously or 1/50 to 1/2 drops injected intravenously or into the perfusate caused a marked degree of recovery provided it was administered before the muscle was entirely fatigued. If the first dose was small and the second ten times greater two consecutive recoveries could be obtained. No difference was noted between direct and indirect stimulation. Guglielmetti also noted that a dose of adrenalin so small as to be unable to influence the fatigue curve may nevertheless render the muscle less sensitive to a succeeding dose.

Okushima (3) likewise worked upon frogs. One muscle was immersed in a solution of adrenalin, the other in Ringer's which served as a control. When the muscles were placed in a 0.0001 per cent solution an increase in the height of the contraction, increased irritability to threshold stimuli, but no change in the contraction curve or in fatigability took place. The "treppe" was also much steeper and of shorter duration in the exposed muscle. A 0.001 per cent solution decreased the excitability of the nerve-muscle but had no effect upon the curve of contraction or fatigability. After the muscle was poisoned with a 0.01 per cent solution it no longer responded to indirect but responded actively to direct excitation.

By direct stimulation of curarized muscle he found no difference with solutions of adrenalin until 0.01 per cent was reached, when the contractions diminished. He believed that there was some antagonism between curare and adrenalin and that adrenalin acted upon the nerve endings.

By means of electric stimuli de Fonseca (4) exhausted the gastrocnemius muscles of frogs and found that by injecting adrenin into the tissue mass the muscle could be immediately restored. When curare was injected simultaneously with adrenalin the former at once lost much of its effect. From these results and those of other investigators he accepts the theory that adrenin neutralizes the products of muscular activity preventing their curare-like effects.

In a recent publication Hartman, Waite and Powell (5) confirm the works of the earlier investigators that animals without the adrenal glands are capable of doing less work and conclude that epinephrin increases the ability of the animal to do work and withstand fatigue. Their experiments do not confirm those of Stewart (6).

Martin and Armitstead (7) noted that adrenalin increased the resting metabolism of the frog's sartorius muscle.

METHOD. The animals, cats, were anesthetized with ether or urethane (2 grams per kilo body weight by stomach) after which they were tracheotomized. Sherrington (8) shielded electrodes held in position by paper clips were applied to both anterior tibial nerves (peroneus communis). The tendons of both anterior tibial muscles were isolated, cut, and to each was fastened firmly a linen ligature some centimeters long. Each leg was bound to the animal board by strong cords one about the hock the other around the foot just below the fastening of the tendon.

The blood pressure was recorded from the right carotid artery by means of either a membrane or a mercury manometer. A chronometer which indicated in most cases intervals of 30 seconds but in a few cases 5 seconds was used.

Two light muscle levers were mounted upon a heavy iron upright with an extra heavy iron base. The magnification of the contraction by the levers was five times for the fatigued and three and one half times for the non-fatigued muscle. In every case the muscles were after-loaded by 50 grams. Above each lever was arranged a delicate pulley over which rode the linen thread which fastened the tendon of the muscle to the writing lever.

The stimulating current was a maximum break induction shock. The primary current was broken by a wheel interrupter. The wheel, 6 inches in diameter, made partly of brass and partly of fiber, was rotated at a uniform speed by an electrically-driven motor. This apparatus through a series of pulleys was designed to interrupt the current from one to several hundred times a minute. The secondary poles of the inductorium were connected to the shielded electrode on the right anterior tibial nerve. The stimulating current for the non-fatigued muscle was also a maximum break induction shock from an inductorium interrupted at a constant speed by a mercury key. The secondary poles of this inductorium were connected to the shielded electrode on the left anterior tibial nerve.

Through a cannula placed in the right external jugular vein the adrenalin[2] was injected usually rapidly, within 15 seconds, but in some cases over a period of 3 minutes. The concentration and quantity of the solutions injected were 1 ml. and 5 ml. 1:100,000; 5 ml. 1:10,000 and 5 ml. 1:1000.

[2] In most cases adrenalin chloride but in a few experiments alkaloid adrenalin (Parke, Davis & Co.) were used.

The blood pressure style, muscle levers and the time marker were placed in a vertical line on the kymograph surface. The rate of the drum was always slow and the muscle contractions were recorded close together 36 to 220 contractions per minute for the fatigued muscle. The non-fatigued muscle was stimulated three times at intervals of 10 seconds and then allowed to rest for 1 minute. The heights of the three contractions were averaged to find the normal.

Frogs of the species Rana catesbiana were also used. The adrenalin was injected through a cannula placed in the left brachial vein. The apparatus, except that that for recording blood pressure was omitted, was the same as that used in the experiments on cats.

RESULTS. *The action of adrenalin upon non-fatigued resting muscle.* Oliver and Schäfer (9) are the only investigators who have studied the action of adrenalin upon the intact resting muscle in mammals. They injected suprarenal extract intravenously into a dog and found that the contractions after the injection were higher and of longer duration than those before. Schäfer (10) in his later works expressed the opinion that the results obtained by him with Oliver were probably not the results of adrenalin but due to impurities contained in the extract.

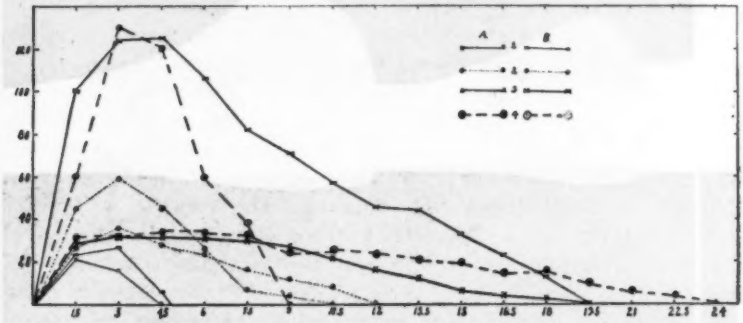
In these experiments adrenalin, injected intravenously, produces in the intact, resting muscle of the cat an effect dependent upon the concentration of the solution in the circulating blood reaching that organ. In this respect it acts the same upon both the non-fatigued and fatigued muscles. In all concentrations used, adrenalin produced a marked increase in the height of muscular contraction and a decrease in the threshold stimulus (increased irritability), of the resting nerve-muscle when occasionally excited. In not a single instance was I able to get a decrease in the height of contraction with doses up to 5 ml. of a 1:1000 solution of adrenalin injected intravenously. The betterment varied from 2 to 100 per cent above the original height. See figures and curve 1. These results confirm Oliver and Schäfer's (9) original work that adrenalin when injected intravenously increases the height of contraction of the resting muscle in the mammal when excited electrically.

In the frog both Oliver and Schäfer (9) and Boruttau (11) noted an increase in the height of contraction and a retarded relaxation with weak solutions of suprarenal extract when injected subcutaneously into the lymph sac or into the circulating blood. The latter observer also noted a complete cessation of contractions when very strong solutions of the extract were injected either into the lymph sac or into the abdominal vein. My results upon the frog confirm their findings.

Inasmuch as the action of adrenalin is the same on both non-fatigued and fatigued muscles a more detailed presentation of the results on the non-fatigued muscle will be given in connection with the fatigued muscle.

The effect of massive doses of adrenalin upon the height of contraction of both non-fatigued and fatigued muscle. The variation in the results of the early observers was probably due to the lack of uniformity in the strength of the solution used.

Cannon and Nice (12) and Gruber (13) tested the effect in cats of very slowly injecting intravenously 2 ml. of a 1:100,000 solution of adrenalin upon the fatigued muscle. They obtained a marked improvement in the height of contraction of the fatigued muscle. Cannon and



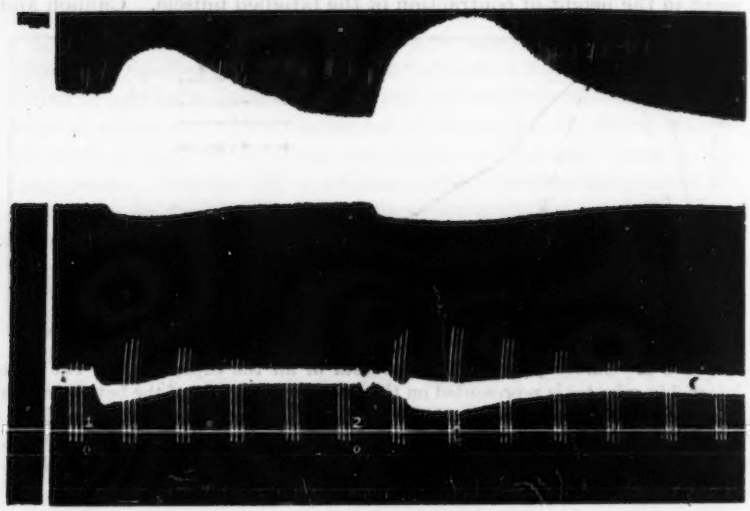
Curve 1. Curves plotted from the data of all the experiments. The time interval in minutes is represented on the abscissa; the value of the betterment in the height of muscular contraction in per cent is represented on the ordinate. *A*, Non-fatigued muscle; *B*, fatigued muscle. The average results from injecting, *1*, 1 ml. of a 1:100,000, and *2*, 5 ml. (1:100,000); *3*, 5 ml. (1:10,000); and *4*, 5 ml. (1:1000) adrenalin solutions.

Gruber (14) published a single record in which 1 ml. of a 1:10,000 solution of adrenalin, slowly injected, caused a marked decrease in the height of contraction while the muscle was being stimulated 300 times a minute. They attributed the decrease, rightfully I believe, to the asphyxia produced by the marked constriction of the arterioles caused by the substance.

Massive doses of adrenalin rapidly injected, intravenously, produce a marked increase in the height of muscular contraction (2 to 240 per cent). (See figures and curve 1.) This increase is, within limits, directly proportional to the concentration of adrenalin in the circulating blood, the degree of decreased irritability of the nerve muscle (fatigue), the rate of stimulation and the condition of the animal. Either very

small or no betterments in the height of muscular contraction are obtained in poor, sickly or confined animals, but enormous increases in the height of muscular contraction are observed in both fatigued and non-fatigued muscles of healthy, well-fed and nourished animals.

The figures presented in this paper were selected from a large number of experiments as typical of the action of this substance upon both non-fatigued and fatigued muscle. Figures 1 and 2 are records taken in sequence from the same animal showing the effect of increasing the concentration of adrenalin in the blood upon the neuro-muscular system. In figure 1 at *1* 1 ml. of 1:100,000 solution of adrenalin chloride was



A B

Fig. 1. Upper record, right anterior tibial muscle excited indirectly 36 times a minute lifting a load of 50 grams. Below it the blood pressure. Middle record that of the non-fatigued muscle. Below it, *O*, zero blood pressure line. In this and all following figures the records read from left to right. Unless otherwise stated the rate of stimulation and weight lifted is the same in all records and the time in half-minute intervals. The horizontal line drawn on all fatigue records shows the degree of rebound of the lever below the base line. Reduced one-third. *A:* Highest contractions before fatigued; *B: 1*, adrenalin chloride 1 ml. 1:100,000; *2*, 5 ml. 1:100,000.

injected intravenously in less than 15 seconds. As a result the blood pressure was increased temporarily 10 mm. of mercury which dropped quickly 50 mm. at which level it remained for some seconds. The maxi-

mum muscular betterment of the fatigued muscle was 40 per cent and only 5 per cent less than its initial height at *A*. The muscle remained above its previous height for 7 minutes. Simultaneously with the betterment in the height of the contraction of the fatigued muscle, the non-fatigued muscle's height was increased by 35 per cent. The muscle remained above the normal for 4½ minutes. At *2* in the same figure 5 ml. of a 1:100,000 solution of adrenalin was injected within 15 seconds. The main effect on the blood pressure was a drop of 40 mm. of mercury. The contractions, however, were increased in height in the fatigued

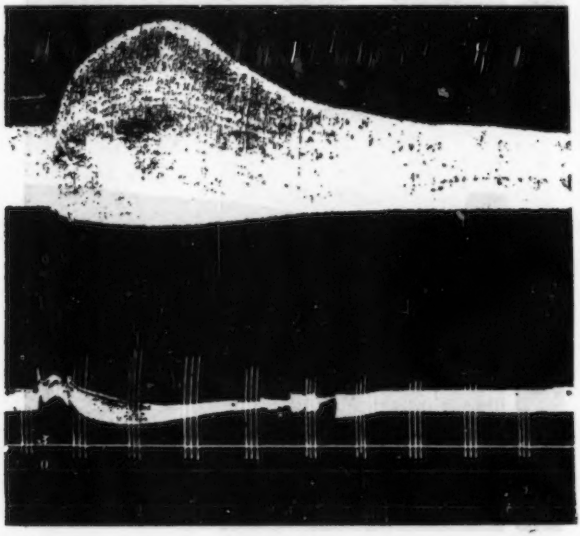


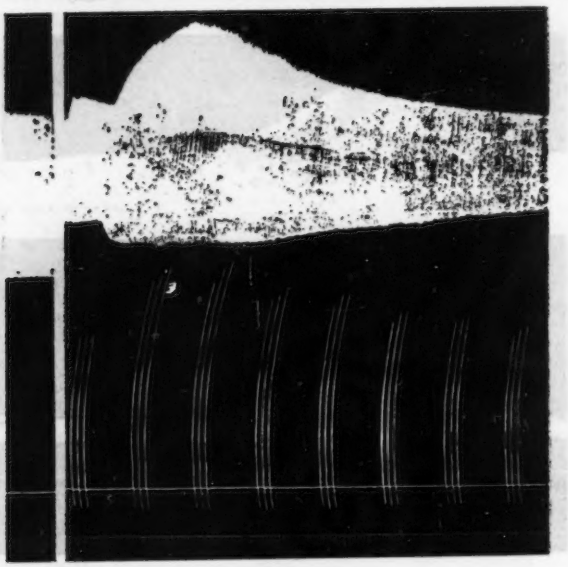
Fig. 2. Same animal as in figure 1. *3*, 0.5 ml. 1:10,000 adrenalin chloride; *x*, clot in cannula removed. Reduced ½.

muscle by over 114 per cent, or 16 per cent over the initial height of muscular contraction, and in the resting muscle by 100 per cent. The betterment lasted for 10 minutes in the former and for 13½ minutes in the latter.

In figure 2 the same quantity was injected intravenously in 15 seconds as in figure 1, *2*, but in a much more concentrated solution (0.5 ml. of a 1:10,000). There was an increase in blood pressure of 40 mm. of mercury which immediately dropped 10 mm. followed by a prolonged fall to 20 mm. of mercury less than the original height. Both muscles

showed increases in irritability and contractility, the fatigued muscle 140 per cent, the non-fatigued 83 per cent. Again the fatigued muscle after adrenalin showed an increase of 16 per cent over the initial height. See figure 1, *A*. The duration of the increased height of contraction in the former was 11 minutes, in the latter, 12 minutes.

Figures 3 and 4 are records taken consecutively from another animal. In figure 3, *B*, at *3*, 5 ml. of a 1:100,000 solution of adrenalin was injected intravenously in 15 seconds. An increase in the height of con-



A B B

Fig. 3. Conditions same as in figure 1 but another animal. Blood pressure was recorded but not shown in figure. It was 142 mm. of mercury when the experiment started and 127 mm. at the end of figure 4. *A*, normal height of muscular contraction before fatigue; *B*, *3*, 5 ml. 1:100,000 adrenalin solution. Time in 5 seconds. Reduced ½.

traction is observed in both muscles. In the fatigued muscle the betterment was 114 per cent requiring about 9½ minutes before the former level was reached. The height of contraction of the non-fatigued muscle was increased 42 per cent. Ten minutes elapsed before the nerve-muscle returned to its former lowered contractility. The betterment

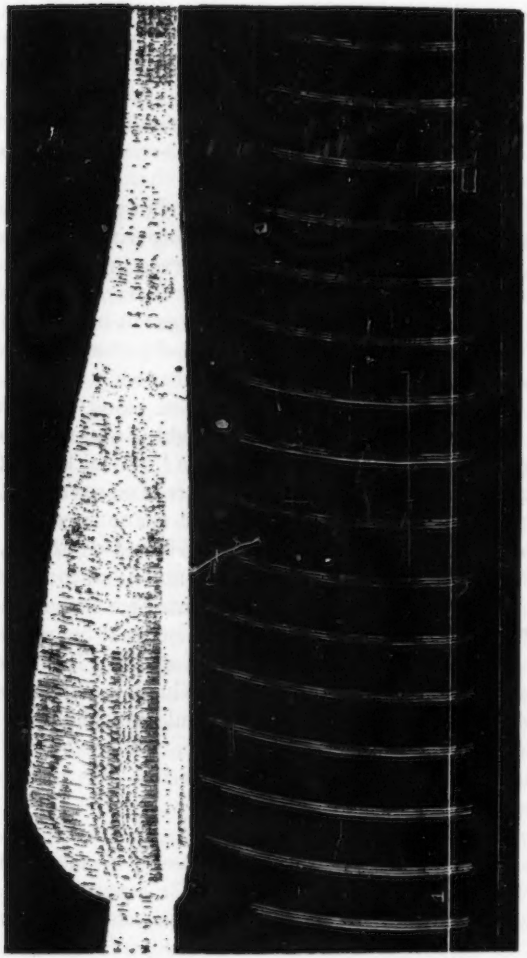


Fig. 4. Same animal as in figure 3. *1*, adrenalin chloride 5 ml. 1:10,000. Reduced ½.

produced in the fatigued muscle was 33 per cent higher than the initial height of contraction of the muscle. See figure 3, *A*. In figure 4, at *1*, 5 ml. of 1:10,000 solution of adrenalin were rapidly injected intravenously. The resultant betterment in the height of muscular contraction in the fatigued muscle lasted about 15 minutes with a maximum increase of 143 per cent. This increased height equaled the initial height of muscular contraction before fatigue. The non-fatigued nerve-muscle showed a maximal increase of 31 per cent. The increase in contractility was demonstrated to have existed as long as that found in the fatigued muscle. Although the blood pressure is not shown in the figures it was recorded by a mercury manometer. The changes in blood pressure were very much the same as those that were observed in figures 1 and 2.

Is the betterment in muscular contraction due to increased blood pressure? By some critics it may be said that the betterment in muscular contraction here shown is due to the increase in blood pressure. In a previous communication (14) the author pointed out that when the blood pressure is above 90 to 100 mm. of mercury increased blood pressure of 60+ mm. of mercury has little effect upon the height of muscular contraction. If, however, the blood pressure is below the "critical level" (90 to 100 mm. of mercury) an increase in blood pressure is more efficient in bettering contraction. In figure 1 in the above communication the blood pressure was 120 mm. of mercury. This was increased 62 mm. by stimulation of the spinal cord, nevertheless the muscular contractions were increased only 8.4 per cent. The betterments here shown after adrenalin varied from 2 to 200 per cent when the original blood pressure was 130 to 160 mm. of mercury. See figures 1 and 2. In figures 3 and 4 it was 142 and 127 respectively. Moreover, in many instances the blood pressure fell and remained down which would preclude any possibility of its being the main factor in bettering muscular contraction. The effect of increased blood pressure must therefore play but a minor rôle in bettering muscular contraction.

Is the betterment due to vasomotor changes within the muscle itself? Kaufmann (16) demonstrated that the vessels of active muscles are dilated. Hoskins, Gunning and Berry (17) reported that adrenalin produces active vasodilatation of muscle vessels and attributed the betterment in the height of muscular contraction from adrenalin to the betterment in circulation. Hartman and McPhedran (18) likewise showed that adrenalin in weak solutions caused vasodilatation of muscle vessels. It was shown by Gruber (19) that adrenalin brought about a betterment in the height of muscular contraction of from 38 to 2730

per cent in muscles in which active vasoconstriction was produced by the substance and that the action of adrenalin did not depend upon betterment in blood flow. Erlanger and Gasser (20) question the possibility of adrenalin causing dilatation of muscle vessels and conclude from their results that "vaso-constriction of both the somatic and the splanchnic areas is the main if not the only effect of the continuous injection of adrenalin." They used, however, extremely large doses, 6 to 11 ml. of a 1:1000 solution, much larger than any of the above observers, who noted vasodilatation. The constancy with which 0.1 to 2 ml. of a 1:100,000 solution of adrenalin produces vasodilatation in muscles in which the nerve is not injured or in which the nerve had been cut and time, 3 to 15 days, allowed for the vessel to regain its tonus, leads one to believe that the methods used by the various experimenters were fairly free from "ambiguities" and vasodilatation is as much a characteristic action of adrenalin in weak solutions as vasoconstriction is in large doses. Immediately after cutting the nerve Gruber (19) found that even weak solutions of adrenalin cause vasoconstriction of the muscle vessels. His results were confirmed by Dale and Richards (21). Cannon and Gruber (14) noted a marked decrease in muscular efficiency as a result of decreased circulation, after large doses.

Figures 5 and 6 are cited as illustrating this effect. In figure 5 the curve is very much shortened because of the extreme vasoconstriction in the muscle. The muscle was quickly asphyxiated as can be seen in the curve when it is compared with figure 4. At *3*, 5 ml. of a 1:10,000 adrenalin solution were slowly injected intravenously. The blood pressure rose from 136 to 214 mm. of mercury (78 mm.). The muscular contraction from the break shock increased 110 per cent in height while the make shock which was subminimal became submaximal and the contractions resulting from it increased from 0 to 53 mm. in height. At *4* the same amount and strength of solution was injected rapidly. The blood pressure mounted quickly from 110 to 212 mm. of mercury (102 mm. increase), with no effect upon muscular contraction. The extremeness of the vasoconstriction can thus be seen. The blood pressure although suddenly increased 110 mm. of mercury was unable to dilate passively the constricted vessels sufficiently to force even a small quantity of blood into the muscle and thus relieve the asphyxiation. This record also shows contracture which is rarely seen in mammalian muscles undergoing fatigue.

In figure 6, *B*, at *1*, 5 ml. of a 1:10,000 solution of adrenalin were rapidly injected intravenously. The muscle improved in height of contrac-

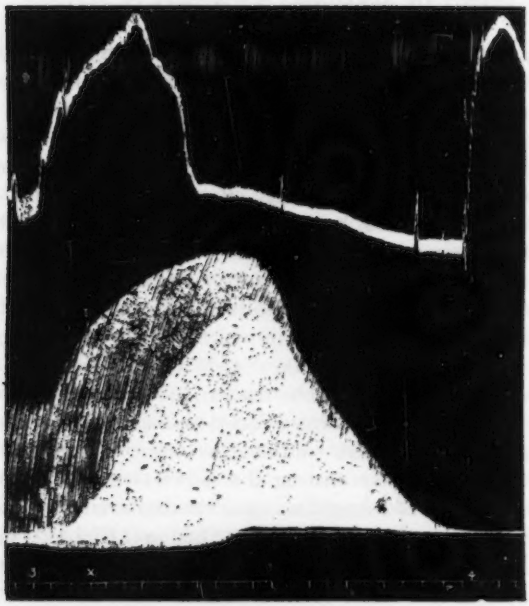
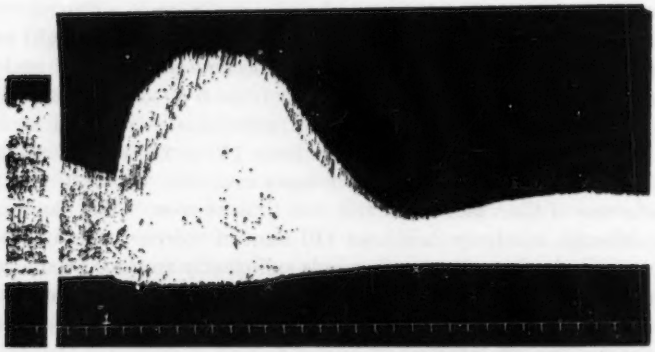


Fig. 5. Upper record blood pressure with mercury manometer. Fatigued muscle curve shows both make and break shock contractions after slow injection of 5 ml. of a 1:10,000 solution of adrenalin at *3* to *x* (marked above time line). *4*, Same quantity and strength of solution injected rapidly. Reduced ½.



A B

Fig. 6. *A*, contractions before the muscle was fatigued; *B*, *1*, 5 ml. of a 1:10,000 solution of adrenalin injected rapidly intravenously. *x*, Lowered contractions due to vasoconstriction. Both make and break shocks produced contractions after adrenalin. Reduced ½.

tion by 135 per cent which was 28 per cent higher than the original initial contraction before fatigue at *A*. The make shock also became effective and continued so during the remaining portion of the experiment. That the betterment in the functioning of the muscle was performed in spite of vasoconstriction and asphyxia is seen in the figure at *x*. The muscular contractions quickly diminished in height the same as in figure 5 but the vasoconstriction gradually disappeared and the muscular contractions improved as seen in the curve. In some cases the blood pressure in the limb was prevented from increasing by pulling on a string looped loosely around the abdominal aorta; in other instances as soon as the muscle began to increase in the height of contraction the femoral artery was ligated; in still other experiments the animal died shortly after the injection of adrenalin. Nevertheless these various procedures had little or no effect upon the initial betterment in the height of muscular contraction. The duration of the improvement was shortened in most cases. Inasmuch as adrenalin produces active vasoconstriction and not vasodilatation in stimulated muscles the main betterment in muscular contraction must be due to other causes than a betterment in circulation.

The effect of adrenalin upon the make shock response. In perfusion experiments with adrenalin it has been a fairly common observation that the make shock contraction which was subminimal before the injection of adrenalin often became submaximal or even maximal in effect. This is not so common in the intact animals because the doses previously injected were very small. With larger doses the make shock can very readily be increased from subminimal to maximal in effect. See figures 5 and 6. In the animals used in securing these records 5 ml. of a 1:100,000 solution caused similar increases in the make shock contraction but to a less degree.

The threshold stimulus of the non-fatigued muscle. Okushima (3) remarked a lowered threshold after adrenalin in resting frogs' muscles. Gruber (21) was unable to note any change in the threshold stimulus in cats after an injection of 0.1 ml. of a 1:100,000 solution of adrenalin. This inability to secure a lowered threshold in resting muscles was due to the fact that small quantities of such dilute solutions slowly injected are very fleeting in action. With larger doses rapidly injected a marked increase in the irritability of the quiescent muscle is noted. The duration of the increased irritability is dependent upon the quantity of adrenalin injected. The larger the quantity and, within limits, the more concentrated the solution the more permanent is the betterment

thus secured. In one denervated muscle a decrease in irritability was observed after the injection of adrenalin.

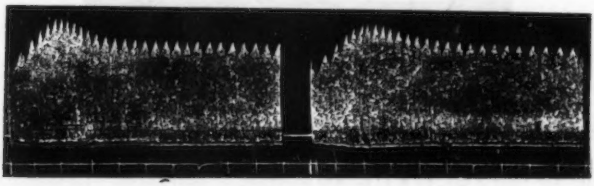
Duration of betterment caused by adrenalin. The duration of the improvement by adrenalin is dependent upon the rate of injection and quantity injected, the rate of stimulation, the weight to be lifted and the condition of the animal. A small quantity of a dilute solution has little effect. The average period of betterment resulting from 1 ml. of a 1:100,000 solution was 4½ minutes while 5 ml. of the same solution rapidly injected produce a betterment lasting for more than 10 minutes. See curve 1. Five milliliters of a 1:10,000 solution rapidly injected caused an average improvement of 19½ minutes' duration and 5 ml. of 1:1000 solution caused in the non-fatigued muscle an average improvement lasting for 24 minutes. The average duration of the fatigued muscle as a result of the latter injection was only 9 minutes. See curve 1.

Generally the non-fatigued muscle shows a somewhat longer betterment in contraction than the fatigued muscle, though the per cent increase is less. This betterment seems to depend upon the concentration of adrenalin reaching the muscle and the rapidity of oxidation of the substance within the body.

What factors alter the results by adrenalin? The curve of betterment is varied by changes in the rate of injection of adrenalin, in the rate of stimulation and in the weight to be lifted. In nearly all cases, especially with solutions stronger than 1:100,000 if injected slowly, the curve develops its maximum gradually and fatigues quickly as seen in figure 5. The rapidity with which the fatigue develops is due to the fact that this concentration produces a strong vasoconstriction, which is enough to counterbalance the effect of adrenalin and the muscle quickly asphyxiates as a result of lack of blood flow. However, when the injection is made rapidly so that practically the full amount of adrenalin reaches the muscle at once a betterment usually out-lasting the vasoconstriction is produced. This does not seem to be the case in the non-fatigued muscles although the betterment seemed to be greater in those cases in which adrenalin reached the muscle in a fairly concentrated solution. Inasmuch as increased rate of stimulation and increased load to be lifted hasten fatigue in the normal muscle the same is to be expected in the fatigued muscle after adrenalin. This is especially true in those cases where vasoconstriction is very decided. See figures 4, 5 and 6. The rate of stimulation in 5 and 6 was twice that in 4 because the make shock became effective. The length of the betterment is thereby very

much shortened. The author was able to confirm the observations of Cannon and Gruber (13) who found that 1 ml. of 1:10,000 solution of adrenalin slowly injected produced a decrease in the height of muscular contraction when the muscle was stimulated 300 times a minute with an initial tension of 120 grams. The results seem to warrant the conclusion that the betterment in muscular contraction is mainly dependent upon the concentration of the adrenalin in the blood reaching the muscle. See curve 1.

Does adrenalin neutralize or destroy the so-called fatigue products of muscular contraction? Most observers believe that adrenalin neutralizes or destroys the fatigue products (1). Few think it increases irritability. Dr. Walter B. Cannon and I, while working together several years ago,



A B

Fig. 7. Muscle stimulated 120 times a minute working against a spring having an initial tension of 120 grams. *A*, Muscular contractions after a rest of 5 minutes; *B*, rest of 5 minutes. Four and one-half minutes before the record was made 1 ml. of a 1:100,000 solution of adrenalin was injected intravenously.

noticed that if adrenalin (0.5 ml. to 2 ml. of a 1:100,000) was injected intravenously while the animal's muscle was at rest, upon stimulation 5 minutes after the injection there was no difference between the response of this muscle and that of a muscle which had merely rested. Figure 7 is such a curve. In figure 7, *A*, the muscle rested for 5 minutes after which it was stimulated for 5 minutes at 120 stimuli per minute contracting against a spring having an initial tension of 120 grams. *B* was taken after injecting 1 ml. of a 1:100,000 solution and the muscle similarly treated. No change is observed in the two curves. This can be explained, I believe, upon the present observations that the action of adrenalin is only transitory. See curve 1. The effect of 1 ml. of a 1:100,000 solution lasts on an average of $4\frac{1}{2}$ minutes. If, therefore, the muscle is stimulated 5 minutes after the injection no change in the height of contraction should be expected. If the muscle is stimulated

2 minutes after the injection the betterment produced will be approximately the same as that found in the continuous curve at the end of 2 minutes. Experiments were performed in which the animal's muscles were rested for a given interval and adrenalin solution injected at varying periods of time before the muscle was again stimulated, as seen in figure 8. In some other experiments the stimulation was stopped while the muscle was responding to adrenalin and 30 seconds later the muscle was again stimulated rhythmically as in figure 9.

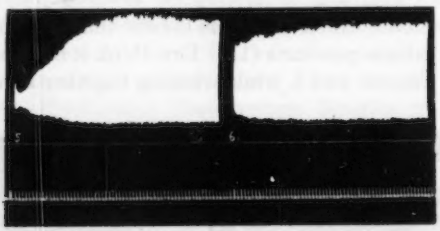
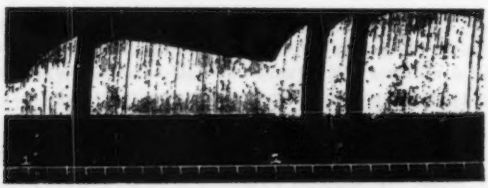


Fig. 8. Urethane anesthesia. Muscle stimulated 128 times a minute working against a spring having an initial tension of 100 grams. *5*, rest 5 minutes; *6*, rest 5 minutes plus 2 ml. of a 1:10,000 solution of adrenalin. Time in 5 seconds.



A B C

Fig. 9. Same as figure 8 except another animal and a rate of stimulation 36 times a minute. *1*, 2.5 ml. adrenalin 1:10,000; *2*, 3 ml. adrenalin 1:10,000. Stimulations stopped for thirty seconds at *a*, *b* and *c*.

In figure 8 at *5* the muscle was rested for 5 minutes; at *6*, 5 minutes' rest was allowed and 2 ml. of a 1:10,000 adrenalin solution were injected intravenously. It will be seen that in *6* the "treppe" was very much altered and the early contractions were much higher than at *5*, in which the muscle was rested only. In figure 9 it will be seen that although the muscle rested 30 seconds at *a*, *b* and *c*, the general contour of the curve was retained. The muscles in both instances were working against a spring having an initial tension of 100 grams with an increase

of 10 grams for each centimeter that the writing point wrote vertically on the drum's surface. In figure 8 the muscle was stimulated 128 times a minute; in figure 9, 36 times.

The fact that the action of adrenalin is merely a temporary action upon the fatigued muscle, and the fact that it has the same action upon non-fatigued muscle seem to support the inference that adrenalin plays no rôle in neutralizing or destroying the fatigue products, but increases the irritability of the nerve-muscle preparation.

SUMMARY

1. Adrenalin injected intravenously has the same influence upon non-fatigued as upon the fatigued muscle. It lowers the threshold (increases irritability) and increases the height of muscular contractions.

2. Massive doses of adrenalin (5 ml. of a 1:100,000; 1:10,000, or 1:1000 solution) rapidly injected produced in my experiments an increase in some cases as much as 240 per cent in the height of muscular contraction in the fatigued muscle and 100 per cent in the non-fatigued muscle when injected intravenously in the intact animal. The increased height in the fatigued muscle was in some instances 33 per cent higher than that of the original initial contractions before fatigue.

3. The increase in the height of contraction is dependent, within limits upon the concentration of adrenalin in the circulating blood, the rate of stimulation and the condition of the animal.

4. The average duration of betterment in the height of muscular contraction was, in the fatigued skeletal muscle, 4 minutes and 40 seconds, 10½, 19½ and 9 minutes respectively from 1 and 5 ml. of 1:100,000 solution; 5 ml. of 1:10,000 solution; and 5 ml. of 1:1000 solutions of adrenalin rapidly injected intravenously. In the non-fatigued muscles the durations of betterment for these same solutions were 4½, 12, 19½ and 24 minutes respectively.

5. Solutions of adrenalin injected slowly do not produce the prolonged betterments seen when the same solutions are injected rapidly.

6. The effects of adrenalin are only slightly, if at all, due to changes in blood pressure or bettered circulation.

7. Adrenalin must be present in the circulating blood through the muscle at the time that the muscle is contracting in order to produce its best results.

8. Adrenalin does not neutralize or destroy the fatigue products but increases the irritability of the muscle whether fatigued or resting.

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FURTHER STUDIES ON THE HYDROGEN ION CONCENTRATION OF HUMAN SWEAT

G. A. TALBERT

From the Hull Physiological Laboratory, University of Chicago

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Historical. What little work has appeared in the literature in regard to the reaction of the sweat seems to be mainly simple qualitative tests. In fact, Kittsteiner (1) was the first to determine the acidity by titration.

The reports of different investigators have been conflicting and even confusing where litmus paper has been employed. Favre (2) and some years later Trümpy and Luchsinger (3) reported that the sweat was normally alkaline. Camerer (4) and Argutinsky (6) obtained variable reactions, while on the other hand Heuss (5) and Kittsteiner maintained that the sweat was normally acid.

The work on animals seems to have been confined mainly to the sweat of the horse and the sweat produced on the cat's paw by electrical stimulation of the sciatic. The former has been particularly studied by Moriggia (7), LeClerc (8), Smith (9) and Pugliesse (10) all of whom agree that only alkaline reaction can be obtained.

Trümpy and Luchsinger and more recently Kittsteiner have made more serious attempts to ascertain the reaction of human sweat.

The former investigators seemed to be impressed with the idea that as normally secreted the sweat was alkaline, in support of which they have cited an experiment in which the palm of the hand was cleansed with ether and tested with litmus paper, with the result that the first secretion was alkaline, then a little later acid, due, as they claimed, to fat decomposition. While no sweat glands are found here, they very discreetly urged that under certain fixed conditions the sweat glands might function after the form of the sebaceous glands. Such claims, however, must be treated as a mere hypothesis and lacking in substantial support.

On the other hand, Kittsteiner reported results quite to the contrary. He maintained that when we had a weakly acid sweat it was only with difficulty that it could be transformed to alkaline, and this change he

claimed was due to bacterial decomposition. In support of his views he took samples of sweat in closed vessels and placed them in an incubator at 37°C. and found that the sweat was still acid after twenty-four hours, and it was two days before it became neutral and six days before it became alkaline, at which time the sample exhaled an unpleasant odor.

Both of these sets of investigators agreed that the sweat was eventually alkaline. There is this difference, however: Trümpy and Luchsinger claimed that the sweat is alkaline in the very act of secreting, while Kittsteiner maintained that it is never secreted alkaline but that the transformation takes place upon standing. Here we find a decided difference in opinion as to the time when the sweat is alkaline and, what is more to the point, as to the causes of the alkalinity.

It resolves itself to a point of interpretation as suggested by Trümpy and Luchsinger; that either we must be dealing with a normal alkaline secreting sweat that becomes acid due to fatty decomposition of the sebaceous secretion or we have a normal acid secreting sweat which only becomes alkaline due to ammonia formed by urea decomposition.

The first alternative is according to the ideas of Trümpy and Luchsinger, the second is that of Kittsteiner.

Whichever theory we accept there is one advantage that Kittsteiner had over all the other investigators: he attempted to determine the acidity by quantitative methods.

Experiments.—In the present work I followed the same method of acidity determination as reported in my first paper (11). The skin was always well cleaned with soap followed by distilled water, ether and alcohol applied with dental napkins. With these precautions I almost without exception obtained an acid-reacting sweat from practically all parts of the body. So by more modern methods and over one hundred tests my results verify those of Kittsteiner and others who maintained that the sweat is normally acid.

In my earlier experiments I found unexpected results, in that the sweat secreted as a result of heat gave a higher acidity than that secreted by work.

In all of these experiments the subjects were naked. The sweat produced by work was by the use of a stationary bicycle in a room at a temperature from 25°C. to 30°C. The heat sweat was produced while the subject was enclosed, except his head, in an ordinary sweat cabinet. The heat was generated by a household electric heater over which was supported a photographer's developing pan containing water for the purpose of producing humidity. The average temperature inside the cabinet was about 40°C.

It was through the suggestion of Professor Carlson that I commenced a series of experiments to ascertain, if possible, the cause of the difference between the hydrogen ions of heat and work sweat.

My problem was to find out whether the sweat glands, in their act of secreting, were actually eliminating more acid by heat than by work or whether it were possible that the very environment of the body, excluding temperature, might be the all-important factor, as the lack of ventilation and the higher humidity of the cabinet compared with the subject working in a large well-ventilated room.

In order to meet these factors it became necessary to contrive a means by which the parts of the body from which the sweat was to be collected should be under the same environmental conditions, as near as possible, except temperature, when acquiring both the heat and the work sweat.

To this end a rubber jacket was so constructed that there would be a hole at the top for the passage of the head, small enough when on to fit snugly around the neck, and likewise was so constructed that it would fit tightly around the waist and upper part of the arms so that part of the body would be enclosed fairly air-tight. The lower border of the jacket was tucked in all of the way around so as to make a trough for the collecting of the sweat. The trough was inclined ventralward on either side in order to cause the sweat to flow into a large test-tube which had been inserted in an opening in the trough. With this method it was not uncommon to collect 50 cc. in less than a half-hour.

A sleeve jacket for the arm was constructed on the same general plan, fitting tightly at each end so as to exclude the air but not to compress unnecessarily the blood vessels. A like jacket was used for the leg. So it was possible to collect sweat from three enclosed parts of the body independently at the same time, and even from an uncovered part as the face or the other arm or leg, as the case might be. So in each experiment it was possible to collect from two to four samples from as many parts of the body with simultaneous sweating. Instead of using the sweat cabinet, as I had previously done for the heat–sweat experiments, the subject occupied a large drying oven where the ventilation was better and proved to be more comfortable.

For the work I used a stationary bicycle which was so geared that it would register miles. While I was not able to ascertain the absolute amount of work the subject did, I was aware of the relative amount as he pedaled for each experiment the same number of miles in the same time, which was six miles in about twenty-five minutes. The work, however, was much more fatiguing than would be the case on an ordinary bicycle traveling on the level through the same distance.

If under these conditions we consult table 1 we will discover that the sweat was collected simultaneously from four parts of the body, viz.: bare face, covered chest, covered arm and covered leg, by means of heat. In table 2 the same parts of the body were employed, but the sweating was the result of work.

These figures show very definitely that the disparity which had been so noticeable hertofore between heat and work sweat as to acidity has now practically disappeared.

The face which gave an average 5.99 pH from heat gave 5.94 pH from work. The covered chest gave an average pH of 5.72 from heat and exactly the same from work. The covered arm yielded an average pH of 5.71 from heat and a pH of 5.73 from work. The covered leg as the result of heat gave a pH of 5.51 and as the result of work a pH of 5.48.

TABLE 1

Heat sweat pH values

FACE	COVERED CHEST	COVERED ARM	COVERED LEG
5.9	5.3	5.33	5.1
5.9	5.75	5.7	5.5
6.0	5.8	5.8	5.7
6.1	5.85	5.8	5.6
6.2	5.85	5.8	5.6
5.9	5.7	5.7	5.5
6.0	5.8	5.8	5.6
5.99 av.	5.72 av.	5.71 av.	5.51 av.

TABLE 2

Work sweat pH values

FACE	COVERED CHEST	COVERED ARM	COVERED LEG
5.9	5.7	5.75	5.5
6.2	6.0	6.0	5.8
6.0	5.8	5.6	5.5
5.7	5.5	5.0	4.8
5.9	5.6	5.45	5.3
5.94 av.	5.72 av.	5.56 av.	5.48 av.

In table 3 are results from samples collected simultaneously from the covered arm and covered leg, and we note here again a marked uniformity as shown for the arm an average of 5.50 pH from heat and 5.58 pH from work. From the covered leg in both instances we obtained the same pH average of 5.39.

In table 4 are given the results of the bare face and covered chest. The average pH value of the face is 5.99 from heat, while the average from work is 6.07. The chest yielded from heat an average of 5.68 and 5.55 from work.

These tables show a remarkable closeness in averages from the heat and work sweat. Even if there is any difference in the averages it is as apt to fluctuate one way as the other.

In table 5 are the results of entirely heat-sweat experiments. The samples were taken from covered chest, covered arm, covered leg and bare leg.

The averages here bring out two quite interesting points. In the first place it will be noted from this set of experiments that there is a regional

TABLE 3

HEAT SWEAT pH VALUES		WORK SWEAT pH VALUES	
Covered arm	Covered leg	Covered arm	Covered leg
5.7	5.2	5.7	5.5
5.3	5.7	6.0	5.8
5.2	5.0	5.6	5.5
5.3	5.1	5.0	4.8
5.7	5.5	5.4	5.3
5.8	5.7	5.7	5.4
5.8	5.6		
5.8	5.6		
5.75	5.3		
5.45	5.3		
4.95	4.85		
5.8	5.6		
5.7	5.5		
5.8	5.6		
5.5 av.	5.39 av.	5.58 av.	5.39 av.

TABLE 4

HEAT SWEAT pH VALUES		WORK SWEAT pH VALUES	
Bare face	Covered chest	Bare face	Covered chest
6.5	6.1	6.6	6.2
6.1	5.6	6.1	5.5
5.6	5.4	6.0	5.2
5.7	5.3	6.1	5.5
5.7	5.3	5.9	5.7
5.9	5.9	6.2	6.0
6.0	5.8	6.0	5.8
6.1	5.85	5.7	5.5
6.2	5.85		
5.99 av.	5.68 av.	6.07 av.	5.55 av.

TABLE 5

HEAT SWEAT pH VALUES			
Covered chest	Covered arm	Covered leg	Bare leg
5.8	5.75	5.3	5.8
5.6	5.45	5.3	5.8
5.5	4.95	4.85	5.35
5.6	5.45	5.3	5.8
5.8	5.7	5.5	5.9
5.7	5.6	5.4	5.7
5.9	5.7	5.3	5.8
5.8	5.7	5.4	5.75
5.8 av.	5.54 av.	5.26 av.	5.73 av.

variation, as is shown by the highest hydrogen ions from the covered leg, this is followed by the arm and with it a fairly close agreement in the chest, while the lowest acidity comes from the bare leg.

The second point to be noted is the difference in the acidity of the covered and the bare leg, which furnishes an illustration of a well-controlled experiment as the sweat from both legs was collected simultaneously.

The same two points are developed in table 1, with the exception that the covered chest and covered arm run very close in their averages, which are 5.72 and 5.71 respectively.

In this instance the bare face, as the exposed part of the body, gave a lower hydrogen ion.

It seems to me these experiments solve the problem. They show why in my earlier observations I obtained higher hydrogen ions from the heat sweat. The results were not due to the sweat being more acid when secreted, but they were more than likely due to the fact that when the subject was enclosed in a cabinet with much humidity and poor ventilation, there was a hindrance to the volatilization of the acid-producing substances in the sweat, which, on the contrary, would take place more easily when the subject is riding on a bicycle with the skin exposed directly to a normal atmosphere.

That there are such volatile substances I am well convinced, as I had reported in my former paper (11).

This showed there were two possible factors to cause this acidity: Either we were dealing with CO_2 or volatile organic acids, or possibly both.

It seemed at first that CO_2 might be a factor, as it is generally agreed that more or less of this gas is being eliminated by the skin. The amount reported has been quite variable in the hands of different investigators. I undertook, however, a series of experiments for the purpose of testing the CO_2 content of the sweat by the Van Slyke method. The collecting tubes in each instance were quickly stoppered after the mouth was withdrawn from the collecting troughs and remained so until ready for the tests, which were made as soon as possible. My results showed that CO_2 was quire a negligible factor.

In all of my testing for the hydrogen ions the test-tubes were freely exposed to the air, so that the CO_2 equilibrium would soon take place, but notwithstanding this fact the acidity of the sweat remained quite the same after hours of such exposure.

After boiling some sweat Kittsteiner claimed that instead of a decrease in acidity he had found, on the contrary, an increase, which certainly throws CO_2 out of the reckoning.

With these results I am thoroughly convinced that the volatile organic acids are at least a factor in the acidity of the sweat. Whether they are the all important factor I am not, at present, prepared to say.

Unna (12) claims that formic, acetic, propionic, butyric and caproic acids may be found.

In the middle of the nineteenth century, Favre believed he had found lactic and what he called hidrotique acid. As to the former, I made some twenty tests with negligible results.

Conclusions. As to the general effect of the rubber jackets, my data show quite definitely that they had a tendency to raise the hydrogen ions of the work sweat rather than to lower them in the heat sweat.

In 53 heat sweat experiments while the subjects were naked in the cabinet, a pH average of 5.73 was obtained, while in 46 experiments enclosed in rubber jackets the heat sweat gave an average of 5.64. These averages are fairly close for so many experiments. On the other hand, 53 work experiments with the naked body gave a pH average of 6.22, while in 28 work experiments with rubber jackets the pH average was 5.65.

This is as we would naturally expect, for the difference in the environmental conditions was much more marked in the two sets of work experiments than in the two sets of heat experiments.

Perhaps what is still more convincing is the grand average of all work experiments with rubber jackets, which gave a pH of 5.65, and likewise all of the heat experiments with the jackets yielded a grand total of pH 5.64.

While it may be true as maintained by Kittsteiner that the higher the temperature under which sweat is produced the higher the acidity, so it may be that the heat is a contributing factor to the higher hydrogen ion value obtained from heat sweat in my earlier experiments. It does not mean that if the heat and work sweat are produced under the same atmospheric conditions there would be any disparity in the hydrogen ions. As a matter of fact, in the data that I am reporting in this article where the parts of the body were enclosed, it appears quite to the contrary. Furthermore, I found on several occasions when the room temperature was quite alike the two kinds of sweating produced about the same averages. Further work may throw additional light on the subject.

The statement which is so often found in the texts, that the acid reaction of the sweat is secondarily due to the fatty decomposition of the sebum, is rather unwarranted or at least is lacking in substantial support.

As to the regional variations, my results are quite contrary to those reported by Kittsteiner in which he speaks of the arms as the most acid, then followed by the face, while the leg was almost constantly neutral. In all my experiments where arm and leg were enclosed, the latter yielded the higher hydrogen ions.

SUMMARY

1. When sweat is secreted under the same environmental conditions, except temperature, the work and heat sweat gave the same hydrogen ions.

2. The sweat collected from the skin covered with a rubber jacket yielded higher hydrogen ions because there is not quite the freedom for the escape of the volatile organic acids.

3. There is a regional variation in the amount of acid eliminated by the sweat glands which is difficult to explain.

I wish to take this opportunity to convey my sincere thanks to Professor Carlson for his helpful suggestions and kindly interest.

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ESTIMATION OF CARDIAC VOLUME IN THE LIVING ANIMAL BY MEANS OF THE TELEROENTGENOGRAM

JOHN H. SKAVLEM

From the Laboratories of Anatomy and Physiology of the University of Wisconsin

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This study comprises a comparison of the volume of the heart of the dog as computed from the x-ray silhouette in the living animal and as measured directly after death and removal from the body. An accurate determination of the heart volume in life has considerable experimental and clinical significance. Bardeen[1] has established the fact that the volume of the human heart can be computed by the x-ray method with an error not exceeding ± 10 per cent and has offered a formula for the computation of the volume from the area of the x-ray silhouette.[2] The purpose of the present work was to determine the formula for the dog's heart, and to establish the fact that variations in area of x-ray silhouette are of significance in that they are determined by corresponding variations in volume.

Method. The animal is placed in the ventral prone position with the chest resting immediately upon the x-ray plate holder. The distance from the front of the chest to the film is only the thickness of a thin aluminum plate and an intensifying screen. The distance from the target to the film is one meter.

By such a position a clear-cut shadow of the heart is obtained which can easily be traced on all its borders. The dog's heart is usually suspended in the thoracic cage so as to be actually free above the diaphragm, so there is not the difficulty in following the diaphragmatic border of the heart such as is encountered in the silhouette of the human heart. The base or upper border of the heart is completed by continuing the line of the right border across the top in a curve to join the line of the left border. After outlining several silhouettes no difficulty is found in drawing in this border with constant accuracy.

[1] Bardeen, C. R.: Amer. Journ. Anat., 1918, xxiii, 423.

[2] Bardeen's formula for the human heart is $V = 0.53A^{3/2}$, where A is the area of the cardiac silhouette.

The measurement of the silhouette area is accurately and easily made by a planimeter. The measurement however must be reduced for the error made by the divergence of the x-rays in their travel from the target to the film. This error is corrected by a simple mathematical procedure. The distance of the film from the target being one meter, it follows from mathematical deduction that

$$A:B::X:Y$$

Where A = size of x-ray silhouette area
B = actual heart area
X = distance of film from target
Y = distance of heart outline from target

The last measurement was made from the plane of greatest area of the heart as determined after the thorax was opened.

One exposure was made with the dog alive and in the position described. The exposures were sufficiently long (2 to 3 seconds) to include several cardiac cycles, and thus give the diastolic silhouette. The exposures were all made at the height of inspiration. The animal was then killed by inhalation of chloroform and another picture immediately taken with the dog in exactly the same position. This was done to ascertain any change in the heart size after death and so control our figures as obtained in measuring the removed heart. The thorax of the animal was then opened and all the vessels of the heart carefully tied so as to maintain the same heart volume after removal. The heart was removed and its volume measured by displacement in water.

Results. The constant to be used in the computation of the volume from the silhouette area was established after a mathematical procedure. The factor 3/2 was chosen to reduce percentage of error. The measurement of each silhouette area was multiplied by this factor and then by dividing with the figure for the actual heart volume the constant for each case was obtained. From the series of twelve cases the constants were averaged and the common constant 0.44 established. The formula as determined is $0.44\ A^{3/2}$ in which A represents the silhouette area.

Table 1 shows the comparison of the volume as computed from this formula with the actual heart volume as measured, with a column showing the per cent of error. The table also shows the change in heart size as revealed by the silhouette area before and after death by chloroform. The change is found to be very slight so that the sil-

houette area of the heart after chloroform death practically corresponds to the area of the heart in normal diastole during life.[3]

Seven of the estimates are too large and five are too small. The greatest percentage error is 10 per cent too small in case 6.

TABLE 1

Computed heart volume and actual heart volume

EXPERIMENT NUMBER	SILHOUETTE AREA		COMPUTED VOLUME $0.44\ A^{3/2}$	ACTUAL VOLUME	PER CENT ERROR
	Alive	Dead			
1	35.2		92	98	6.6−
2	27.1	29.0	69	70	2.0−
3	67.9	66.1	236	230	2.8+
4	35.5	36.2	96	95	0.8+
5	89.3	88.3	364	325	7.1+
6	31.2	32.2	81	90	10.0−
7	39.2	40.6	113	108	5.1+
8	49.0	49.8	153	140	9.5+
9	39.2	38.8	108	110	2.2−
10	35.1	34.1	91	95	4.2−
11	30.6	33.8	86	85	1.4+
12	38.7	38.4	105	98	6.8+

TABLE 2

Heart weight to body weight

	CASE											
	1	2	3	4	5	6	7	8	9	10	11	12
Body weight in kilos...	9	5.3	24.2	7	19.5	6	6.5	11.3	7.1	7	6.7	7.1
Heart weight in grams.	68.3	46.5	156.5	54	217	65.7	69	108	77.5	75.2	62	75
Per cent of body weight	0.75	0.87	0.65	0.77	1.11	1.09	1.06	0.95	1.09	1.9	0.92	1.07

Relation of heart weight to body weight. Data were also gathered in this series of animals concerning relation of body weight and heart weight. Table 2 shows this relation.

The per cent of body weight for the heart in the dog is somewhat higher and far more variable than in the human. Table for these cases

[3] That this is by no means true after death from other causes has been found by Meek and Eyster in this laboratory in work on asphyxiant gases, ordinary asphyxia and ether.

shows the per cent to vary from 0.65 per cent to 1.11 per cent and to be constantly higher than 0.5 per cent which is about normal for the human heart. While one can estimate with fair accuracy the "normal" or "average" size heart for a given body weight in man, this is obviously impossible in the dog.

The relation of the volume of blood and of tissue to the total volume of the dog's heart. After death the vessels were tied, the heart removed, and the volume of the entire organ measured by immersion in water. The vessels and heart chambers were then opened, the contents removed and the volume of the muscle measured by immersion in water. From these figures the heart chamber volume is readily computed and the percentage volume of muscle and chambers determined as shown in table 3.

TABLE 3

Heart muscle and heart chambers volumes

	CASE											
	1	2	3	4	5	6	7	8	9	10	11	12
Heart muscle volume	60	45	150	55	225	60	68	90	70	65	55	68
Per cent of muscle to total volume	61.2	64.2	65.2	56	68.9	66.6	61.9	64.2	63.6	68.4	64.7	69
Heart chambers volume	38	25	80	40	100	30	40	50	40	30	30	30
Per cent of chambers to total volume	38.8	35.8	34.8	44	31.1	33.4	38.1	35.8	36.4	31.6	35.3	31

SUMMARY

1. The relation of the volume of the dog's heart to the area of the x-ray silhouette made in the frontal plane is expressd by the formula $0.44\ A^{3/2}$, in which A represents the area of the silhouette. Volume determinations made in this way are probably accurate within 10 per cent.

2. There is no consistent relationship between heart weight and body weight in the dog. The size of the heart can therefore not be inferred from the size of the body, as in man, but must be measured.

3. The relation of volume of the cardiac chambers to the whole organ appears to be a fairly constant one. In the heart after death by chloroform, a little more than one-third of the total volume is made up by blood contained in the chambers, a little less than two-thirds of the total volume is due to the muscle.

STUDIES OF CELL DIVISION

I. The Effect of Dilute Sea-Water on the Fertilized Egg of Echinarachnius parma during the Cleavage Cycle

E. E. JUST

Rosenwald Fellow in Biology, National Research Council

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Several workers (1), (2), (3), (4), (5), (6) have demonstrated with various methods that in the egg of *Arbacia* there is a rhythm of susceptibility to toxic agents that accompanies the first cleavage cycle. Chief among these are Mathews (2) and R. S. Lillie (5) who have likewise formulated theories of cell division based on their results. During the summer of 1920 at the Marine Biological Laboratory, Woods Hole, Mass., the writer (7), while investigating the very short period following insemination during which the egg of *Echinarachnius parma* is markedly susceptible to dilute sea-water, found it advisable for comparison to study the effects of dilute sea-water on the egg through the cleavage cycle. The present paper embodies the results of this study.

I. When inseminated eggs of *Echinarachnius parma* are exposed to the action of dilute sea-water they take up water and swell; in sea-water of slight dilution they reach a point of equilibrium without disintegrating. They remain thus for a longer or shorter time which depends upon the degree of the dilution of the sea-water. If the dilution be great (30 per cent of sea-water and less) the eggs swell and cytolyze. The rate of this cytolysis in a given dilution of sea-water is most rapid in eggs that are exposed to the dilute sea-water just before cleavage. The period of susceptibility of the *Echinarachnius* egg to dilute sea-water is thus similar to that found by R. S. Lillie (5) in the egg of *Arbacia*.

The rate of cytolysis of eggs while in dilute sea-water constitutes an excellent criterion for determining this period of susceptibility in the developing egg. The results are sharp, well-defined, and admit of little dispute. This is particularly true if the cytolysis comes on rapidly; for this reason I used 100 per cent tap-water in most of the

observations. Again, study of the rate of cytolysis involves observations on individual eggs during the process of disintegration—a method which has been fruitful of interesting results. Thus, with it the writer has established the fact that during the period of membrane lifting the egg passes through a very critical period as shown by its high degree of susceptibility to dilute sea-water. The value of this method is also seen at once if among eggs of a given lot there are individual variations with respect to the cleavage rhythm. For example, after the first cleavage the difference in cleavage tempo of a lot of eggs increases making it difficult to obtain clear-cut results on eggs *en masse*. But it is very simple to mount such eggs under the microscope and watch them individually.

To the use of tap-water for determining the susceptibility periods in the cleaving eggs, there is, of course, a certain objection; it is a brutal method, not capable of nice results. The situation, therefore, calls for a method that sharply marks off the periods of susceptibility—a dilution that has no very serious effect on the egg except during these periods. An approximation of this is to be found in the dilutions of sea-water around 50 per cent. They form a check on the results obtained by tap-water.

I summarize now experiments on the rate of cytolysis of the developing egg while in tap water which show the periods of susceptibility.

Eggs inseminated in sea-water. At 2 minute intervals drops of these eggs are exposed to tap-water and the rate at which they cytolyze while in the tap water recorded. In ten experiments eggs reach the height of resistance 26 to 32 minutes after insemination. At this time they withstand tap-water treatment for 300 to 330 seconds. At 40 to 43 minutes after insemination resistance drops—time to cystolysis is 30 seconds. Seventy-three to 84 minutes after insemination the egg is extremely susceptible—time to cytolysis is about 20 seconds. Ninety-five to 98 minutes after insemination (first cleavage) the egg is resistant—time to cytolysis is 120 to 130 seconds.

Experiments in dilute sea-water (60 parts tap-water plus 40 parts sea-water) give comparable results. Eggs in which one or more blastomeres cleaved before others—especially eggs in the eight cell stage at the time of micromere formation—likewise show a susceptibility before cleavage. One can, therefore, obtain very pretty demonstrations of the separate rhythms of cell division in the blastomeres of the cleavage stages. The method is thus differential and may be capable of precise use in the study of cleavage problems.

A variation of the method so far considered is to obtain the per cent of cytolysis of the eggs after a given number of minutes in the dilute sea-water. Thus, eggs are inseminated in sea-water and at 2- or 5-minute intervals they are transferred to dilute sea-water up to and through the first cleavage. Then after 20 or 40 minutes the per cent of intact eggs in the various dishes is taken. A number of experiments was made in this way both with tap-water and with different dilutions particularly that made up of 60 parts tap-water plus 40 parts sea-water. In eleven experiments of this group the eggs were found susceptible at 40 to 44 minutes after insemination and again about 10 minutes before cleavage.

What is true of the first cleavage is also true for the second. The susceptibility is therefore a rhythmical phenomenon bound up with the cleavage process.

We may correlate the results obtained by the study of cytolysis in dilute sea-water with another method which briefly is as follows:

Eggs are inseminated in normal sea-water and at intervals transferred to hypotonic sea-water from which after 1 minute they are removed to normal sea-water and their development noted. One minute should be sufficient for the exposure since during the critical period the eggs (as shown above) while in the dilute sea-water cytolyze in less than 1 minute. The object is to give a short exposure that will just cover the critical period. By counting about three hundred eggs in each exposure one gets a good percentage index for the critical period. This method eliminates the personal factors as far as possible and the results are fairly reliable.

11:10 a.m. Two lots of eggs from two females, each lot in 10 cc. of sea-water. Eggs in good condition without a trace of accidental insemination, not a single membrane formed.

11:20 a.m. The two lots of eggs mixed and inseminated with light sperm suspension in 300 cc. of sea-water. One drop of these eggs pipetted off at 2-minute intervals to 10 cc. of tap water where they remained for 1 minute, then collected in a pipette and transferred to 10 cc. of sea-water. Controls: uninseminated in sea-water; inseminated in sea-water.

2:00 to 4:00 p.m. Counts of cleaving eggs made. Eggs exposed 38 minutes after insemination gave 30 per cent cleavage. Eggs exposed during the period 89 to 90 minutes after insemination gave no cleavage.

Results with dilute sea-water (60 parts tap-water plus 40 parts sea-water) give comparable results. Eggs are also shown susceptible just before the second cleavage.

This method can be checked by the estimation not only of the number of eggs that cytolyze but also of the eggs that cleave. The cytolyzed

eggs and the cleaving eggs may be estimated in each dish or separate observations may be made to cover each in turn. Both methods were followed.

A word concerning these observations may not be out of place. The eggs were from fresh animals used as soon as procured, when this was possible. Control eggs were kept inseminated in sea-water to determine the per cent of eggs that would divide under normal conditions; and an uninseminated control to make sure that the results would not be obscured by the mixed conditions in a given lot of eggs due to accidental insemination were always run. *No experiment was ever considered if the control showed less than 95 per cent of development or if the uninseminated control showed a single membrane.* It is possible with extreme care never to have a single membrane formed in the uninseminated control. Nothing so vitiates results as the heterogeneity of the egg population due to accidental fertilization; for then the cleavage which it is desired to have as nearly the same throughout the lot as possible is arhythmic and the observation is really worthless.

The methods of these observations differ somewhat from those used by R. S. Lillie (5) in his study of the resistance of *Arbacia* eggs to dilute sea-water, but the results obtained are of the same kind. My methods emphasize the complete disintegration. In addition, I have merely carried out the simple corollary of estimating the cleavage. He allowed the eggs to remain in the hypotonic sea-water and I transferred mine to sea-water after a minute.

The uninseminated Echinarachnius egg will withstand treatment with tap-water for 2 and in some cases 5 minutes before cytolyzing. Some 10 to 20 minutes after insemination the eggs are even more resistant. A slight drop in resistance comes on midway between insemination and cleavage, followed by a rise in resistance. This in turn is followed by a sharp drop in resistance. Recovery then follows and the egg divides. Any single experiment made would establish this, and the observations together leave no doubt of the fact.

Dilute sea-water is not the only agent that betrays the susceptible period before cleavage. Recently, Baldwin (6) has shown that *Arbacia* egg is susceptible to alcohols. Previously, Moore (4) has shown a diminution in the resistance of this egg to hypertonic sea-water. Finally, the pioneer work in this field by Lyon (1) should be mentioned. Despite the fact, as subsequently shown by Mathews (2), that Lyon missed the exact stage at which the egg of *Arbacia* is least resistant to the action of KCN, this work of Lyon's is as important as

any of the remarkable investigations with which he has enriched the field of experimental embryology. Spaulding (3) likewise demonstrated susceptibility in *Arbacia* by various methods.

Nor yet have observations and experiments been limited to the eggs of echinoderms. Other ova pass through a period of low resistance prior to cleavage. Thus, F. R. Lillie (8) says of the egg of *Nereis* that centrifuging during the anaphase and telephase tends to inhibit cleavage. Conklin (9) using various agents found that the egg of *Crepidula* is not susceptible just before cleavage. It may well be, therefore, that ova generally exhibit rhythmical resistance to environmental changes. If this be true the study of this phenomenon becomes important for any analysis of the physiology of cell division. And indeed, the susceptibility of the *Arbacia* egg at least plays a large part in the formulation by R. S. Lillie (5) of a theory of cell division.

It seems to me, however, that any speculation concerning the significance of the susceptibility period appearing before cleavage is of value only if the period be rather sharply defined. Merely to say that the egg suffers a drop in resistance before the cleavage begins leaves too wide a gap in a process so well ordered as that of mitotic cell division in animal ova. When one considers the whole mechanism of this type of cell division—not merely the mitotic spindle or the cytoplasm alone—and the synchrony of events in division, one must offer a postulate that considers every part of the mechanism. Mathews made, therefore, an important contribution when through the study of sectional material he fixed the susceptibility period of *Arbacia* egg to KCN as the time when the asters are at the height of their development—a finding highly consonant with his theory of cell division.

Thus, from this point of view F. R. Lillie's (8) statement that in the egg of *Nereis* centrifuging during anaphase and telephase tends to inhibit cleavage becomes vastly important. The susceptible period is here clearly defined. In what follows I shall present observations that attempt similarly to fix the susceptibility period in the living egg of *Echinarachnius*. The report on the study of sectioned eggs I must leave to a future date.

II. If on the evidence submitted we concede that the egg of *Echinarachnius* exhibits before cleavage a marked drop in resistance to dilute sea-water we must address ourselves to the query, at just what stage in the cleavage process does this drop in resistance manifest itself. According to Mathews (2) and F. R. Lillie (8) the susceptibility period in *Arbacia* and *Nereis* is well defined. In the egg of *Echinarachnius* the susceptible period is likewise well defined.

Wilson (10), (11), (12) in several places has given descriptions of the normal fertilization and mitosis as seen in the egg of *Toxopneustes;* the events leading to first cleavage in the living egg of *Echinarachnius* are closely similar. In addition I have noted certain changes in the hyaline plasma layer. In *Echinarachnius* the events leading to first cleavage are briefly as follows:

The sperm and its aster move toward the female nucleus. With conjugation of the nuclei the astral rays stream outward reaching the cortex. The hyaline plasma layer now exhibits delicate irregularities in its contour. This "amoeboid" phase becomes more pronounced. The rays die down. There then appears in the nuclear region a clear streak which is the intact zygote nucleus with hyaloplasm substance at the nuclear poles. *At this time the egg is susceptible.* The "streak" grows and widens—so that it is dumb-bell shape. This means that the nucleus breaks down, the nuclear content escapes and the hyoplasm spheres at the spindle poles increase in size. The "dumb-bell" gives rise to a well-defined mitotic complex with hyaloplasm spheres that increase in size and astral rays that are more extensive. The asters are meantime moving apart to their definite positions. The egg is still spherical. Meantime the hyaline plasma layer is again active. With the stage of greatest growth of the hyaloplasm sphere, when the astral rays are very extensive, the hyaline plasma layer exhibits an amoeboid activity from over the poles toward the equatorial zone of the spindle. This results in an accumulation of hyaline plasma layer material in the zone of the future cleavage plane. The egg is most susceptible at the time when the hyaloplasm sphere is largest, the rays extended, and the hyaline plasma layer is about to move toward the equator of the spindle from the area over the spindle poles. When the hyaline plasma layer heaps up at the equator the egg is again resistant. The egg now rapidly elongates in the spindle axis. Beneath the equatorial girdle of hyaline plasma layer a cleft appears, triangular in optical section, which is the beginning of the first cleavage plane. The hyaline plasma layer rapidly moves in and the two blastomeres arise. The period of susceptibility thus falls in the late anaphase and the telephase.

Mathews gives a table which compares his results with those of Lyon on the effect of KCN on the egg of *Arbacia.* There are here also two periods of susceptibility. These coincide with the development of the asters; the period of immunity coincides with the regression of the asters and the development of the nucleus.

Lillie finds the egg of *Nereis* most susceptible to centrifuging during the anaphase and the telephase. In *Crepidula*, according to Conklin, "the greatest changes are produced when the eggs are in some phase of kinesis at the beginning of the experiments, while stages of interkinesis are affected relatively little."

But the data on *Echinarachnius* certainly show that the eggs are susceptible before cleavage at the time when the hyaloplasm spheres are largest, the astral rays widely extended, and the hyaline plasma layer is about to move from the area over the spindle poles to the equator. The amoeboid movement of the hyaline plasma layer certainly shows that it is no mere inert sheath. This behavior of the hyaline plasma layer is not peculiar to the egg of *Echinarachnius*. Thus, Lillie (13) has described amoeboid waves in *Chaetopterus* eggs. The early descriptions of von Erlanger are well known. Observers have described the equatorial girdle of hyaline plasma layer in echinid eggs; McClendon (14), for example, as well as a number of others. Andrews (15) has given a particularly good description of the hyaline plasma layer.

During membrane lifting (16) the egg of *Echinarachnius* cytolyzes by breaking only in that zone from which the membrane is lifting at the time of exposure. At what point does this egg break when exposed to dilute sea-water during the susceptible period before cleavage? The observations give a categorical answer to this question. *In the period before cleavage when the egg of Echinarachnius is susceptible to sea-water it breaks down by bursting over the poles of the spindle.*

If fertilized eggs of *Echinarachnius* in a Syracuse watch of normal sea-water are mounted under the microscope during the susceptible period before cleavage they may be readily observed when dilute sea-water is added to them. If tap-water be added they burst within 15 to 30 seconds. With dilute sea-water (10 to 30 per cent sea-water) the results are about as rapid. With 40 per cent sea-water (60 parts tap-water plus 40 parts sea-water) the eggs do not burst so quickly. In a dilution of sea-water made up of equal parts of tap-water and sea-water, the rate of bursting is slightly lower. With a dilution of 60 parts sea-water plus 40 parts tap-water the eggs tend to form buds rather than to burst.

I employed these dilutions mentioned in order to determine the exact point at which the eggs burst when put in dilute sea-water during the susceptible period before cleavage. In general, less dilute sea-water causes the eggs to form buds; a greater dilution causes them to

burst; and the more dilute, the more rapid the rate at which they burst.

If one depends solely on the use of tap-water for these observations and is slow in adding the tap-water, allowing the eggs to settle, and bringing them under focus, etc., one may miss the process entirely. In all cases observed the eggs cytolyze during the susceptible period just prior to cleaving by bursting over the poles of the cleavage spindle. This is true not only of the first cleavage but of every cleavage studied as far as the blastula stage. The egg never breaks in any other way. In the less dilute sea-water, the cytoplasm bulges over the poles of the spindle. In the more dilute sea-water the break and outflow come on violently; and indeed in many cases it is hard to catch the process so rapidly do the eggs cytolyze.

An egg, then, is susceptible to dilute sea-water prior to cleavage because of a drop in resistance of the egg cortex (hyaline plasma layer) at this time. Such an egg rapidly takes up water and bursts quickly because of the low tensile strength of the hyaline plasma layer in the area over the poles of the spindle.

An egg that has passed the period of susceptibility on exposure to dilute sea-water does not cytolyze by bursting over the poles of the spindle. If such an egg has elongated, it merely takes up water, becomes spherical, and disintegrates without giving evidence of a region of highest susceptibility. If the cleavage plane is forming, this disappears. The hyaline plasma layer heaps up at the cleavage furrow, swells and becomes very conspicuous. The endoplasm rushes forcibly toward the poles—indeed, it gives one the impression of a violent contraction. If the blastomeres are actually apart, each disintegrates separately. Quite frequently in the two-cell stage, one blastomere remains intact and the other disintegrates rapidly; the number of eggs in which this happens is correlated with the number of eggs in which one blastomere is slightly in advance of the other in the cleavage rhythm. When the egg in the two-cell stage reaches the susceptible period just before the second cleavage, the blastomeres rupture over the spindle poles, that is, in the spindle axis parallel to the first cleavage plane.

A point worthy of emphasis brought out by these observations on the egg of *Echinarachnius* (*cf.* too Conklin's (9) observations) is that the egg is susceptible *before* it begins to elongate in the spindle axis and before the hyaline plasma layer has fully heaped up at the site of the future cleavage plane. This would seem to indicate first that

the drop in resistance over the poles of the spindle is not due to the thrust of the spindle. Secondly, the egg does not elongate because of the moving of the spindle poles toward the egg periphery. Rather, the evidence would seem to show that the egg elongates through the force exerted at the equator by the accumulated hyaline plasma layer in this zone.

We may sum up the results of the study of the period of susceptibility which the egg of *Echinarachnius* exhibits just before cleavage as follows: It undoubtedly comes on *a*, during the anaphase and telephase, *b*, while the egg is still spherical and *c*, before the hyaline plasma layer fully heaps up at the equator (site of the future cleavage plane) due to its movement from the area over the spindle poles. This movement brings about a thinning of the hyaline plasma layer in the polar areas. The susceptibility is due to a development of weakness in the cortex over the poles so that when the egg takes up water it rapidly bursts at these points. An earlier period of susceptibility is found just before the zygote nucleus breaks down.

These observations on the egg of *Echinarachnius* showing that in the susceptible period just before cleavage the egg breaks in the area over the spindle poles are by no means new. They are really but a confirmation of some earlier work by Conklin (9) on the egg of *Crepidula*. With various agents he frequently obtained a protrusion of the cytoplasm always from the areas over the spindle poles. This protrusion was most pronounced if the spindle were formed. Conklin, therefore, concluded that in cell division there is a decrease in surface tension over the spindle poles. Now Conklin has also made the interesting observation that the egg plasma may protrude in the area over the future spindle pole before the spindle forms. He therefore reached the important conclusion that the decrease in surface tension in the polar regions is not brought about by "mitotic pressure."

Conklin (17) has likewise described the production of large polar bodies in the egg of *Crepidula* by centrifuging at the time of polar body formation. Now, I think that we may easily explain this. The polar area becomes more fluid due perhaps to activity of cortical substance at the animal poles. The spindle meets little resistance as it moves into this area. If now we centrifuge the egg, it tends to rupture at the animal poles, hence the protrusion of cytoplasm to form a bud larger than the normal polar body. Chambers (18) was able to induce what we might call accessory polar bodies in the polar region of the egg of *Cerebratulus* simply by pricking the egg in this

region; this seems to indicate a greater fluidity here than elsewhere on the egg surface at this time.

As I see it, my observations on the egg of *Echinarachnius*, which show that during the period of susceptibility before cleavage the egg in dilute sea-water bursts over the spindle poles, indicate a drop in the tensile strength by the hyaline plasma layer. At this time the hyaline plasma substance is about to move away from the polar areas to the equator. We may perhaps say that the egg is thus being denuded of hyaline plasma material in the polar areas. It is as though the plasma layer which protects the egg through its presence by thickening down at this time exposes the endoplasm at the poles. Eggs exposed during this period to dilute sea-water take up water—perhaps no more rapidly than at any other period after fertilization. But there is this important difference: the eggs that take up the water during the susceptible period are eggs with local weaknesses. Such eggs burst rapidly in these weakened zones. The egg gives way at the poles because of the activity of the hyaline plasma prior to moving from the poles.

Now Conklin (19) has made an observation that lends credence to this view. According to this worker, new cell walls at division are formed chiefly at the poles. In *Echinarachnius*, certainly, that part of the cell wall (hyaline plasma layer) over the spindle poles moves down to the site of the future cleavage plane and then moves inward to form the new cell walls between the two blastomeres. In the meantime, in the exposed areas over the poles with little or no hyaline plasma layer, new hyaline plasma material is rapidly constituted by the underlying cytoplasm. And it is this reconstitution which makes for a return in resistance.

Finally, I may call attention to the movement of peripheral granules in the egg of *Echinarachnius*. Granules at the equatorial zone early move inward toward the spindle. This is clearly shown in the dumbbell stage. Wilson particularly has called attention to this point. Meves' (20) beautiful figures show the relation of the granules to the spindle area very clearly. In addition, as is well known from the descriptions of Mrs. Andrews, Gardiner, Ziegler, Fischel, McClendon and others, at the time of cleavage granules come to lie inside the cell walls. The classic work on this point is of course that of von Erlanger.

In the egg of *Echinarachnius parma*, then, by means of dilute sea-water we find a period of susceptibility just before cleavage. In this period we are able to correlate the behavior of the hyaloplasm spheres,

the astral rays, the nucleus, the movement of ectoplasmic granules and the amoeboid activity of the cortex. And the behavior of these parts of the cell are but expressions of the rhythmical exchange between nucleus and plasma. In any attempt at the solution of the problem of cell division we must consider this streaming behavior of the protoplasm.

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THE FERTILIZATION-REACTION IN ECHINARACHNIUS PARMA

V. The Existence in the Inseminated Egg of a Period of Special Susceptibility to Hypotonic Sea-Water[1]

E. E. JUST

Rosenwald Fellow in Biology, National Research Council

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In the first paper of this series (1) on the fertilization-reaction in *Echinarachnius parma* the writer called attention to the cortical changes that manifest themselves in this egg as a response to insemination. Briefly, these are as follows:

Soon after it takes in the sperm the inseminated egg lifts off the membrane. This formation of the "fertilization membrane," as it is usually called, is due to a liquefaction throughout the cortex that brings about the formation of the perivitelline space and thus the separation of the membrane. It has often been held that membrane separation is of importance in preventing polyspermy. Now in *Echinarachnius* these observations on the separation of the membrane are of significance because they quite clearly showed that the block to polyspermy is established before the actual separation of the membrane. The change in the egg whereby polyspermy is prevented is of extreme rapidity. But during three summers sufficient observations on individual eggs were made to make clear just what takes place. With care one can observe the actual entrance of the sperm concerned in the fertilization-reaction. It was learned in this way after mixing the eggs and sperm that it takes a certain time for the sperm to reach the egg. This time will vary with several factors: the concentraton, the activity and the age of the sperm suspension, the temperature of the sea water, the vitality of the eggs and the time in the breeding season. These or at least some of these factors will likewise determine the penetration time of the sperm,

[1] The experiments were made at the Marine Biological Laboratory, Woods Hole, Mass., during the summer of 1920. They were reported at the 1920 (Chicago) meeting of American Society of Zoölogists as part of a Symposium on Fertilization. An abstract appeared in the *Anatomical Record* for January, 1921.

that is, the time that it takes the sperm to get into the egg from tip to base. Now, the actual time of penetration of the sperm within the egg—that is, the time elapsing between the moment when the tip of the sperm head touches the egg to that at which the base of the head disappears within the egg—is of extremely brief duration. This time is not easy to measure, except with slowly reacting eggs or sperm when the penetration time is lengthened. Early in the season when the temperature is low one can best follow the penetration. I did not give any figures for the penetration time in the paper cited. I did, however, relate the response of the egg to additional sperm entry to the penetration time of the fertilizing sperm for indeed this was clearly brought out in the obsrvations.

A wave of negativity moves over the egg, its rate varying with the variations in time that mark the disappearance of the sperm within the cytoplasm. And if the penetration is rapid or slow, the response of the egg to additional sperm is of the same order. It must, however, be admitted that there may not be any causal connection between these two phenomena.

In tables 1 and 2 on page 4 of the paper mentioned it is readily apparent that after the sperm is within the egg—regardless of the time the actual penetration may have consumed—there follows now a period of about 15 seconds before membrane separation begins. (Thus, in table 1 sperm are engulfed at 50, 49, 30, 45 and 30 seconds after insemination and the membrane comes off at 65, 64, 50, 67 and 50 seconds respectively after the sperm has disappeared within the egg.)

During this period of membrane separation the egg is extremely susceptible to hypotonic sea-water.

If eggs of *Echinarachnius parma* are placed under the microcsope and inseminated one may observe the rapid penetration of the sperm and with it the wave of negativity as evidenced by the behavior of supernumerary sperm around the egg. There then follows the period of about 15 seconds before membrane separation begins. The moment that the membranes begin to lift the eggs are treated with tap-water. In 15 to 20 seconds after the tap-water is added, the eggs burst. This cytolysis is violent, rapid and complete. But for the use of sea-water of graded hypotony it would have been extremely difficult adequately to interpret this behavior. With the use, however, of sea-water ranging in dilutions from 95 per cent to 5 per cent, the whole period has been clearly revealed. If the egg is treated with tap-water when the membrane is one-third or a half off instead of at the moment of membrane

separation, the result is the same—there is a complete cytolysis in some 15 seconds. If the egg is allowed to separate its membrane from its entire surface except the very last point opposite the site of sperm entry (from which the membrane lifts last) and is exposed at this instance, then cytolysis takes place as before.

The actual course of membrane separation runs over a period anywhere from 9 to 26 seconds. During this period the egg cannot withstand treatment with tap-water with anything like the resistance of the uninseminated egg. Following complete membrane lifting there ensues a period during which the membrane rounds out and becomes equidistant from the egg. The egg is now resistant again although the resistance is not so great as that at about 10 minutes after insemination. But it is clear that with the formation of the membrane and even before its rounding out the egg has recovered its resistance to tap-water. The following summary of experiments reveals these facts.

The uninseminated egg. Uninseminated eggs mounted under the low power of the microscope are treated with tap-water. With a stop watch the rate of cytolysis in 10 lots of eggs each from 10 females is determined. The time in tap-water to cytolysis is as follows:

	NUMBER									
	1	2	3	4	5	6	7	8	9	10
Time in seconds to cytolysis.....	270	243	60	113	150	148	256	247	155	240

The rate of cytolysis, in eggs from these same females, 5 to 10 seconds after insemination, is about the same.

The inseminated egg during membrane separation. Eggs from these same females inseminated in turn and exposed to tap-water during membrane separation gave the following rates of cytolysis:

	NUMBER									
	1	2	3	4	5	6	7	8	9	10
Time in seconds to cytolysis.....	14	17	19	20	9	11	18	16	7	6

The inseminated egg 2 minutes after insemination. Eggs from these same females exposed to tap-water after membrane separation cytolyzed around 120 seconds.

Once it was well established with tap-water that the susceptible period falls in exactly with the period of membrane separation, attention was directed to the susceptibility of the egg to less dilute sea-water at different stages of the process of membrane separation. This seemed important because with the 100 per cent tap-water it appeard that *when the egg cytolyzed the break always came from that part of the cortex from which the membrane was lifting at the time of exposure.* The cytolysis with tap water was, however, far too rapid to be sure of this. The less dilute sea-water proved that this interpretation was correct. *When eggs are exposed to dilute sea-water during the period of membrane separation they cytolyze by an outflow of cytoplasm at the points from which the membrane is lifting at the moment of exposure.* The data on the effects of sea-water of different dilutions are summarized in what now follows:

Eggs were tested during the period of membrane separation of sea-water diluted with tap water to give 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90 and 95 per cent sea-water.

The results with these dilutions may be briefly summarized. In 95 and 90 per cent sea-water the eggs do not show any change. In 85 and 80 per cent sea-water the eggs do not break down but merely show a slight protrusion of protoplasm during membrane elevation. Thus, in 85 per cent sea-water the eggs show 1 per cent buds. The response of the egg during membrane separation to treatment with 75 per cent sea-water is about the same as that to 80 per cent except that the percentage of buds is slightly increased and that the egg may elongate slightly. Eggs kept in these dilutions show some development; the more dilute giving no true cytoplasmic cleavage, the less dilute giving nuclear as well as cytoplasmic division.

Thirty parts tap-water plus 70 parts sea-water: When eggs are exposed to the action of this dilution during the period of membrane separation they form buds. For example, if eggs are exposed at the moment of membrane separation the membrane bulges out at the point of membrane lifting and there follows a protrusion of the cytoplasm beneath the bulging membrane. If the membrane has been lifted from half the egg then the buds form in the angles (i.e., as seen in optical section) between the lifted membrane and the part of the cortex from which the membrane is not yet lifted. If the membrane stands off from the egg at every point except the very last point of its attachment—the pole opposite that of sperm entry—then the bud forms at this point.

Thirty-five parts tap-water plus 65 parts sea-water and 40 parts tap-water plus 60 parts sea-water: Eggs exposed to these dilutions show

changes comparable to those shown in 30 parts tap-water plus 70 parts sea-water, but the changes come on more quickly. Thus, in 70 per cent sea-water the eggs exposed during membrane lifting form buds in from 1 to 1½ minutes; but in 60 per cent sea-water these buds form in about 40 seconds. Moreover, while the percentage of eggs with membranes that respond to the treatment of 70 per cent sea-water with bud formation beneath the lifted membrane is from 70 to 80 per cent, the percentage of eggs with membranes that thus respond to the 60 per cent sea-water is always close to a hundred. Such eggs however tend to disintegrate rather than to form buds. The formation of these buds is always beneath the membrane at the point at which it is lifting. They never arise from the region from which the membrane has lifted or from the region in which the membrane is still stuck to the egg.

Hypotonic sea-water of 55 parts sea-water plus 45 parts tap-water and sea-water of greater hypotony: These dilutions are cytolytic and in them the eggs respond much as do eggs in 100 per cent tap-water except that the onset of cytolysis is not so rapid. They are indispensable for working out the wave-like nature of the cytolysis in the membrane eggs. If eggs are placed in the dilution 40 parts sea-water plus 60 parts tap-water at the moment of membrane lifting they disintegrate instead of budding in the region of membrane separation. At this point the egg protrudes rapidly. This is followed by an outflow of cytoplasm through the region of liquefying cortex. The cytoplasmic outflow results in the formation of a dumb-bell shape mass of egg material within the partially lifted membrane. If eggs are exposed to the action of these dilutions of sea-water after the membranes are one-third or one-half off, then the disintegration takes place in the zones from which the membrane is just lifting. Such eggs exhibit a girdle of protruding cytoplasm within the membrane. In optical section this girdle appears as buds in the angles between the lifted membrane and the egg. The outflow of cytoplasm that leads to disintegration is in this angle around the egg. If eggs are exposed just before the membrane is lifted from the last point of the egg—the pole opposite sperm entry—the cortical break is in the zone immediately around this point. If the eggs are exposed at the very instant that the membrane is lifting from its last point of attachment, the break comes at this point: there is a bud of cytoplasm which precedes the cortical outflow leading to complete cytolysis. Such eggs, therefore, respond like those that receive an exposure at the very beginning of membrane lifting. The two are, however, readily distinguished by the great difference in the extent of completely lifted membrane.

It is thus seen first that the suceptibility of the egg of *Echinarachnius* falls in with the period of membrane separation. But what is more important is that like the process of membrane separation the susceptibility travels at the same rate as the membrane lifting wave. As the cortex goes into solution, thus lifting the membrane,—a process easily followed under the microscope—droplets of ectoplasm move across the perivitelline space before they completely disappear. Any point on the egg surface where this dissolution is taking place becomes the point of susceptibility at the instant of the dissolution.

In the next place, one must note the most remarkable characteristic of this period of susceptibility during membrane lifting. The susceptibility of the cortex is rapidly reversible. I have made observations on thousands of eggs and have yet to see an egg exposed during membrane separation break in those regions from which the membrane has not lifted. This is especially well brought out by exposing eggs during the early stages of membrane separation. Any part of the egg from which the membrane is not lifted is resistant to the dilute sea-water. And it is equally true that any part of the egg from which the membrane has lifted is resistant. This is strikingly shown by exposing eggs just at the moment that the membrane is being lifted from the last point on the egg surface. In this case the breakdown is only at this point; the zones from which the membrane is already off are resistant. When the membrane is fully off, the egg leaves the period of susceptibility. We may say, therefore, that a wave of resistance to dilute sea-water follows in the wake of the wave susceptibility. There is an exceedingly rapid restitution process in the cortex following a momentary loss of resistance through the normal cortical breakdown that pushes off the vitelline membrane.

We may conclude: The period of susceptibility to dilute sea-water exhibited by the inseminated egg falls in with the period of membrane elevation. In eggs exposed during this period the cortex breaks and allows an outflow of cytoplasm, a bud-like protrusion, in the less dilute sea-water; or a more pronounced outflow with complete disintegration in the more dilute sea-water. This break in the cortex takes place only in the zone of membrane separation that is, where the cortex through secretion is lifting the membrane at the instant of exposure The cortex is therefore weakest in the zone of membrane separation and this weakness travels over the egg with the wave of membrane separation. Zones of the egg from which the membrane has lifted are resistant; zones of the cortex to which the membrane is still stuck are resistant. *The suscepti-*

bility of the egg is thus due entirely to the progressive cortical secretion which brings about membrane lifting. It is a manifestation of local (cortical) changes.

These observations are likewise of fundamental significance in establishing that the period of susceptibility is an expression of a period of profound physical changes. A study of these might well furnish us with a means of a closer approach to the fertilization problem. These manifestations are, however, of importance for their own sake.

THE NATURE OF THE PERIOD OF SUSCEPTIBILITY. 1. We may turn our attention first to the rôle of the so-called "fertilization membrane," during the susceptible period. The question as to whether the membrane is preformed or arises *de novo* as the result of fertilization need not seriously concern us here, although it seems to me that the bulk of the evidence indicates in *Echinarachnius* and in *Arbacia* that the membrane is preformed. We should nevertheless remember that in our experiments made to determine the presence of the membrane on the uninseminated egg the very procedure of the experiment itself might be sufficient to call forth membrane separation. The very fact that such diverse agents may cause membrane separation proves or at least suggests that the membrane separation process is highly reactive. Therefore, experiments to show the presence of the membrane of the uninseminated egg may themselves produce the membrane by the action of the agents employed. But this is apart from the question now under consideration; namely, what part does the separating membrane play in the period of cortical breakdown when the egg is so highly susceptible to the effect of hypotonic sea-water?

In the first place, as shown above, the egg may cytolyze within the membrane which remains intact. In the second place, in the uninseminated egg the membrane does not burst but remains intact while the egg substance goes into solution. The effect of the hypotonic sea-water is to harden the membrane so that it stands off from the egg but it does not usually rupture. Since, however, the membrane is evidently undergoing a change in its physical properties just about the time that it begins to lift we should naturally expect the most pronounced change in its behavior at this time. In other words, the membrane may actually play a rôle in the behavior of the egg when subjected to the hypotonic sea-water but this rôle may be so small that it is obscure; the best time to observe any change in the behavior would be at the time when this change is at its height. This would be at the very beginning of the membrane separation process. When the egg, at the moment mem-

brane separation is beginning, is put in the hypotonic sea-water, the membrane bulges out to quite a perceptible degree at this point. This effect as elsewhere noted in this paper could not have been adequately studied had not the hypotonic sea-water been used in closely graded series. With hypotonic sea-water of varying strength this process can be worked out in great detail and is about like this: When the membrane begins to separate at the point of sperm entry and the egg put in hypotonic sea-water, the membrane lifts off from the egg at a greater distance than otherwise. If this hypotony is very great, tap-water for example, the egg bursts. If the degree of hypotony is less, the egg forms a bud at this point. To quote from my notes:

> July 8, 9:20 a. m. Inseminated eggs in sea-water 35 seconds later eggs in 30 tap-water plus 70 sea-water.
>
> First the membrane bulges out at the point at which it starts lifting; this is immediately followed by the budding of the cytoplasm just beneath. This behavior is not so clearly shown in eggs put into the tap-water or hypotonic sea-water when the membrane is off the greater part of the egg. It is best observed in those eggs put into hypotonic sea-water at the very instant that the membrane is beginning to elevate.

I interpret this as follows: The membrane before insemination is a very plastic substance. After it begins to lift or rather at the moment it begins to lift it becomes more hardened; it sets. Before it begins to separate from the egg surface it has the general properties of the cell. But once it separates from the cell surface it is no longer a part of the metabolic machinery. Now during the time that it bulges out from the egg it is in its most elastic condition. As fast as it lifts from the egg it rapidly hardens and loses the properties of the cell protoplasm. The bulging at this time is due to the elasticity which soon passes off.

That something like the above is true seems to be supported from what we know of exovates. The best time to secure exovates is soon after the membrane is formed; after this they are more difficult to obtain. In other words, it is easiest to rupture the membrane soon after it has arisen. Moreover, the best time to shake off the membrane from either inseminated eggs or butyric-acid-treated eggs is immediately after the membrane has separated. This would seem to lend support to the view given above, namely, that the membrane changes its properties after it lifts from the surface of the egg.

The conclusion of this whole matter is therefore that the egg membrane is not a living part of the egg once it is separated from the egg surface and that it plays no rôle in the susceptibility of the egg. The

phenomena osmotic or otherwise go on in virtue of the cell substance and not through or because of the vitelline membrane.

2. If indeed it be true that the membrane once off the egg plays none but a passive rôle in the metabolic activity of the egg, we must conclude that there is some alteration in the egg itself. And this is indeed the case as shown by a comparison of the physical character of the uninseminated and the inseminated egg with a membrane after treatment with hypotonic sea-water.

The uninseminated egg when subjected to the action of tap water becomes granular and disintegrates. At one point in the cortex just under the membrane the cytoplasm forms bubbles. These gradually form throughout the egg. The membrane now stands out as clearly as the so-called "fertilization membrane." After the cortex goes into solution in this way the interior of the egg is destroyed. The cytoplasm resembles crystals of salt through which water is seeping, so granular is its appearance. The effect of the tap water in lifting the membrane is often lost sight of because the swelling of the cytoplasm is so closely connected with the breakdown of the cortex that the water-logged cytoplasm fills out the distended membrane. Disintegration now takes place *within* the membrane. This is the usual procedure. Occasionally, however, the egg ruptures at one point and cytoplasm pours out at this point from the egg. The two striking characteristics of the behavior of the uninseminated egg in response to the treatment with tap-water are the granular appearance of the cytoplasm and the general uniform disintegration of the egg. It is as though the cytoplasm is washed away.

With the inseminated egg during the susceptible period the case is quite different. In the inseminated egg treated with tap-water the egg ruptures at one point and flows out. It may form a ball after it flows out and so maintain itself in sea-water for some time. The cytolyzed plasma tends to cohere. This outflow usually does not pass beyond the membrane which remains intact. Or, if the outflow forces itself through the membrane the rest of the membrane remains intact and spherical. In other words, the physical properties of the inseminated and the uninseminated egg are quite different as shown by the difference in reaction to hypotonic sea-water. This change is apparent the *moment the membrane begins to lift* and can be very clearly revealed by the study of the behavior of the egg in hypotonic sea-water of graded strength.

3. The susceptible period here described is not, however, the only one in the egg after insemination. As is well known particularly from the very careful experiments of R. S. Lillie (2) the egg of *Arbacia* is quite susceptible to hypotonic sea-water just before cleavage. In my study of *Echinarachnius*, in order to get all the data I could I have studied the susceptibility of this egg before cleavage. It is practically like that of *Arbacia*. Just before cleavage a period of great susceptibility to hypotonic sea-water sets in. It was deemed advantageous to study this in detail in order to determine what the two periods have in common. A brief comparison may here be made. In the case of the recently inseminated egg that is susceptible to hypotonic sea-water, the susceptibility is due to the breaking up of the cortex in that part of the egg where the membrane is lifting. In the case of the egg that is susceptible to the hypotonic sea-water just before cleavage the hyaline plasma layer is intact. The conditions therefore are quite different for in the one case we have the cortex breaking up later to build the hyaline plasma layer, in the other we have the hyaline plasma layer already formed. But the susceptibility of the egg at the time just before cleavage is due in part to the changes in the hyaline plasma layer as I have elsewhere shown for at this time the susceptibility is clearly localized over the spindle poles.

4. If we attempt to come to some conclusion as to the meaning of this susceptibility we must take into account the following facts. First, the very rapid recovery of the cortex after membrane lifting. Second, the resistance of the cortex from which the membrane is not yet lifted. Third, the apparent failure of the cortex at the site of sperm to dominate the rest of the cortex in any way, either in greater resistance or susceptibility, or in persistence of the effects of the hypotonic sea-water. In brief, we must keep in mind that this susceptibility is clearly localized; or, what is more correct, that the break in the egg which is the expression of decreased resistance to the hypotonic sea-water is clearly at the site of membrane lifting. Now this does not mean that this is the point at which the water enters the egg. Rather, it means that the point at which the membrane is lifting is the point at which the cortex is weakest. We are not dealing with an imperforate membrane but rather *with the cortex* that is not continuous; it shows a break in the zone of membrane lifting. The normal process of the cortical secretion is doubtless bound up with water movement. The cortex is washed away. The material goes into solution. Now, this process is one of rapid recovery not only in the cortex itself but toward the interior of the egg for the interior

of the egg never becomes involved. In the normal process just enough water is present to carry out the normal process, but when the egg is placed in dilute sea-water, the picture is different. The recovery in the zone of membrane formation cannot take place, and the cortex breaks. Moreover, with the cortex gone, the endoplasm is without the cortical protection and it too becomes involved so that complete cytolysis is the result. We are here dealing, then, with a sensitivity due to actual progressive dissolution of colloids in the cortex of the egg.

5. To the reader it is at once apparent that the process of membrane separation exhibited by the *Echinarachnius* egg closely resembles that of transmission in various tissues. The way in which the cortex responds to hypotonic sea-water may be additional evidence of this similarity. Certainly, the response of the cortex during membrane formation to the hypotonic sea-water reminds us of the action current in a stimulated nerve. The high susceptibility of the cortex in the zone of membrane elevation and the relatively resistant zones from which the membrane has not separated suggest the electronegative condition of a nerve fiber set up wherever the excitation may be at a given instant as it sweeps along the fiber. It may well be that this is more than a superficial resemblance and that the study of this cortical response would warrant further investigation from this point of view.

6. It seems that this wave of lowered resistance in the cortex of the egg during membrane separation is worthy of study as a secretion. Our knowledge of the actual secretory processes in cells as such is not so abundant that we may ignore any process that even merely seems a secretion. The striking cortical behavior of the egg of *Echinarachnius* might, therefore, yield valuable information for the problem of secretion.

These then are some of the points which the study of the susceptibility in the inseminated egg of *Echinarachnius* has raised. For its own sake it will repay further study. Far more significant for us, however, at this time is this question: what is the relation of this susceptibility period of the so-called fertilization-reaction? If the susceptibility period be interpreted in terms of the permeability theory or if it should be found that there are changes in oxidation and in electrical potential at this time, would these findings be interpreted as meaning that the one or the other or all these changes are the "cause" of fertilization? The writer has consistently held the point of view that the essential phase in the whole fertilization process is a reaction which is practically instantaneous between sperm and a substances of the egg, namely, the fertilization-reaction. If therefore, the findings on the

susceptible period be interpreted as the leading event in the whole fertilization process, then the reaction between egg substance and sperm is far from instantaneous but is indeed spread over an appreciable length of time. In a forthcoming paper evidence is presented which attempts to establish that this susceptible period is not the fertilization-reaction, but rather is a local change which is a sequela of the reaction between egg substance and sperm.

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STUDIES ON THE PHYSIOLOGY OF CAPILLARIES

V. The Reaction of the Human Skin Capillaries to Drugs and other Stimuli

E. B. CARRIER

From the Laboratory of Zoöphysiology, Copenhagen University

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Until the last few years all that was known of the physiology of the small vessels in the human skin had to be inferred from the color changes and general condition and temperature of the skin, from blood pressure fluctuations, and from what might be assumed by analogy from observations made on animals, such as the frog. When Lombard (11) in 1912 found that a drop of oil on the human skin made it possible, with the aid of a strong light, to see microscopically the blood flow in the skin vessels, he opened this field for the direct examination of both the physiologist and pathologist. Lombard's simple technique consisted in placing a drop of glycerine or transparent oil on the skin, illuminating with a Nernst lamp, and examining the field with a Greenough binocular microscope, magnifying from 10 to 25 times. He reports that he was able to see "a beautiful picture of the papillae and of the superificial blood vessels. . . . . Each capillary loop seemed to spring from a more or less circular little mound. The mounds were the papillae, the tissue of which was so transparent that the red capillaries within stood out strongly." He finds a more striking picture at the base of the nail, where the loops may be seen in profile as "exceedingly delicate reddish threads, which have a wavy, often twisted, course."

In 1916 Weiss (21), from the clinic of O. Müller, Tübingen, published the first of a considerable series of articles on microscopical examination of the capillaries in man. His technique in principle is the same as that of Lombard. From this clinic has come the description of a microscopical tube, fitted with an electric light and battery and adapted for observation of the skin on any part of the body (22). Some of the most recent papers from Müller's clinic, containing reviews of the previous work, are those by Weiss and Holland (23) and by Parrisius (14), (15).

Schur (16), (17) and Niekau (13) have both given an illustrated histological description of the skin and its vessels and have compared this with the skin and capillaries as seen in the living subject under the microscope. Schur has suggested the use of a small cover glass, 10 mm. square, to prevent the blinding reflection of the light from the uneven surface of the oil.

Since this microscopic study of the capillaries was commenced at Tübingen, the study of the skin and its vessels in disease has been carried on with enthusiasm. Skin diseases, nephritis, heart insufficiency, diabetes, aneurisms, arterial sclerosis, some acute infections, vasoneurosis and thyrotoxicosis are some of the diseases investigated by this method. Each new worker has preceded such observations by a description of the normal vessels, especially with respect to their size, shape and the character of the stream flowing through them. With the aid of higher magnification, it has been possible not only to see, as Lombard did, the red of the blood in the capillaries and underlying venous plexus, but also to observe the flow in the capillaries, to note the rate of the current, distinguish red and white cells, to observe the state of contraction of the vessels, and to see the so-called granular stream and those gaps in the stream, which have been compared to peristaltic waves.

This paper concerns itself only with the morphology of the capillaries in health and their reaction to certain drugs and other stimuli.

Method. For source of illumination an arc lamp was used, because of its bright light and comparatively large field. The light was filtered through a solution of copper sulfate and picric acid. This filter removed both the heat rays and the violet rays, and had the additional value of making the tissues appear almost clear white instead of red orange, as in the case of unfiltered artificial light. The light was focused with a lens. The oil used was paraffin oil, usually without a cover glass, except when the skin was very uneven. The microscopes were a *Greenough* binocular, with oculars 1 and 3, objective 25 mm. and a magnification of 38 and 61 times, also a *Zeiss* microscope, with oculars 2 and 4 and objective AA magnifying 50 and 90 times. The places which proved most favorable for observation were the base of the nails on the third, fourth and fifth fingers, the back of the hand and, in some cases, the wrist and forearm. In most cases the subject and observer were the same, due to an exceptionally favorable field for examination, and the illustrations are taken from the writer's own hand. A number of other normal subjects were also examined, however.

In describing the appearance of the capillaries at the base of the nail, much has been repeated which already may be found in the literature. The chief justification for such repetition is first, that previous descriptions are printed chiefly in German and may not be so familiar to all English readers; and second, that it has been possible, with the above described light filter and favorable subject, to see more of the connection between the capillaries and larger vessels than has previously been described.

The field at the base of the nail. At the base of the nail the capillary loops, which elsewhere rise almost at a right angle from the underlying vessel plexus to the epidermis, here become gradually more horizontal. In a hand, where the skin has been allowed to grow up onto the nail, there is at last a fringe of long narrow loops with, several tenths of a millimeter proximal, a deeper lying plexus of vessels, receiving the blood from these capillaries. This plexus is a close-meshed richly anastomosing, horizontally lying net-work of vessels, which corresponds very closely with the description of the venous net given by Spalteholz (19). At the base of the finger-nail the appearance is somewhat modified by the fact that the capillaries here are in almost the same plane as this venous plexus.

The most striking feature of the picture at first glance is the variety in shape and size of the capillary loops. At one extreme are vessels so very fine as to allow the passage of only one or two cells abreast. These are usually, though not necessarily, straight regular loops. At the other extreme are large, often very much twisted vessels, several hundredths of a millimeter in width, which may have the form of a single loop or that of a figure 8, or may be greatly twisted and folded on themselves, especially if there has been a little injury to the skin. The length of a capillary loop also varies between wide limits. Bruns and König (1) give a maximum of 0.4 mm., except in cases of nervous patients, when the loops may be longer. As figure 1 shows, this is considerably less than what we have found. The length of the capillaries at the base of the nail is very largely influenced by the type of work for which the hands are used and the freedom with which the skin grows over the nail.

The venous arm of the capillary loop is a little broader than the arterial and may be traced in the large loops to its termination in the underlying venous plexus. In some cases it is possible to see the arteriole supplying two or more loops and thus to trace the course of the blood quite all the way from the arterial to the venous side. (See fig. 1.)

The arrangement of arterioles, so far as can be seen, is similar to that of the venules. A terminal branch finally ends in a capillary loop, giving off in its course one or more other capillary loops. A number of smaller capillaries empty into the venous limb of one large capillary, which, after receiving several tributaries, unites with the underlying venous plexus. As may be seen from the figure, not all the loops

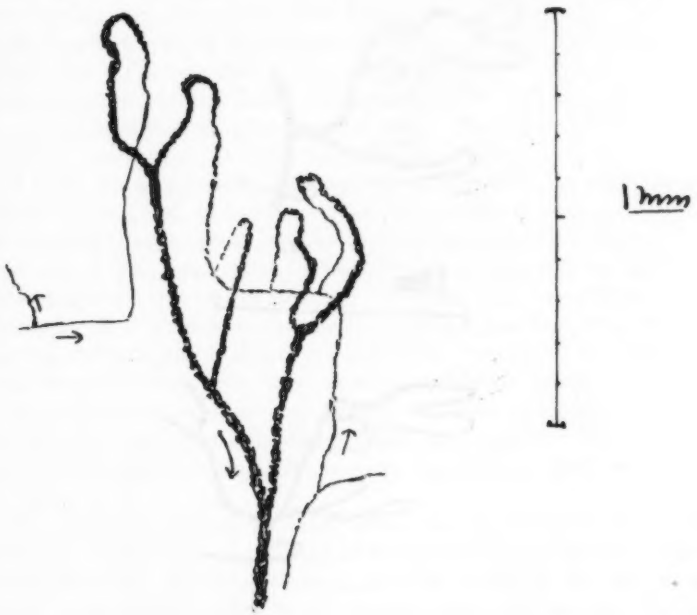

Fig. 1. A group of capillaries from the base of the nail, fourth finger, left hand.

emptying into the same venule need necessarily be supplied by the same arteriole.

The shape and relation of the capillaries remain practically unaltered for a long time. *A* and *B*, figure 2, show the same capillary group with an interval of almost seven months.

By watching the rate of flow of the blood in a group of capillaries coming from a single arteriole, it can be seen that the blood may flow

at the same rate in all, or that its rate may differ greatly in each of the loops; that is, some of the capillaries at a given minute are more contracted than their neighbors, supplied from the same arteriole. Hagen (5) has represented graphically the difference in width of neighboring capillaries. When the stream is rapid it appears almost homogeneous, but in a slow stream the white cells show as little white spots. Often

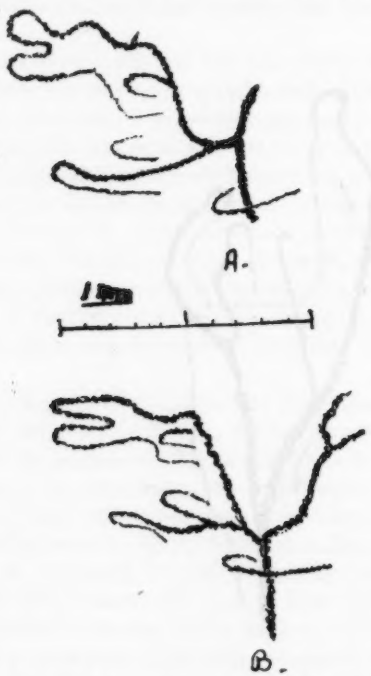

Fig. 2. *A*, a group of capillaries as seen on October 15, 1921; *B*, the same group April 9, 1922.

there is a slow stream which looks granular, due to clumping of erythrocytes, especially under the influence of cold. The rate of the stream varies, in the same capillary, from minute to minute and is very readily influenced by temperature, as will be described later.

Another peculiarity of the stream, which may not be found in all normal individuals, but is certainly present in some, is the occurrence of what has been compared to peristaltic contractions (20). The stream

slows and then starts forward again with a gap, about a tenth of a millimeter in length, inserted in the column of blood. The capillary walls are not visible; so whether this represents a true contraction of the capillary or a peculiarity in the distribution of the cells, is difficult to say from observation only. What is seen is a space free of red blood cells. It has been explained as a contraction wave passing over the capillary and helping thus to forward the blood stream. Hagen, however, makes the observation that during stasis the cells in a capillary draw together toward the center, leaving a gap filled with plasma at either end. This is sufficient to explain the gaps which follow a slow, jerky stream or stasis. It is occasionally possible, however, to see gaps in a stream moving at a fairly rapid rate. That these are not due to capillary contraction, the two following observations indicate. In the first place this gap travels at the rate of the blood stream and so varies greatly in its speed; and in the second place, if the stream is watched in two capillary loops, where both loops come off at the bifurcation of one arteriole, it may sometimes be observed that gaps will travel simultaneously over the two capillaries. This can best be explained by a skimming off of plasma, so that areas of plasma, free from cells, are seen passing through the capillaries. Hence the burden of the proof lies with those who look upon these gaps as contraction waves.

The color of the capillaries is, of course, the color of the blood in them; for it is the red blood cells which make them visible. This is bright arterial red when the circulation is good, but becomes deep venous blue in stasis.

The field on the back of the hand. Here the capillaries are most often seen only at their tips, about 20 or 25 to an area 0.7 mm. square. Sometimes, however, the venous branch may be traced all the way to the richly anastomosing network of vessels below and we can see this horizontally running plexus of vessels receive the blood from the capillary loops at regular intervals. Occasionally the vessel supplying two or more capillaries may also be visible, but only for a very little way.

If a single small area on the back of the hand, about 0.7 mm. square, is observed over a period of several minutes, it will be found that here, unlike conditions at the base of the nail, all the capillaries are not open at a given moment. They are opening and closing continuously. Figure 3 shows the same field at various hours of the day. While all the vessels are not open at one time, certain of the capillaries are almost always open, while others open only occasionally. This was substantiated by comparative drawings from other areas also. The

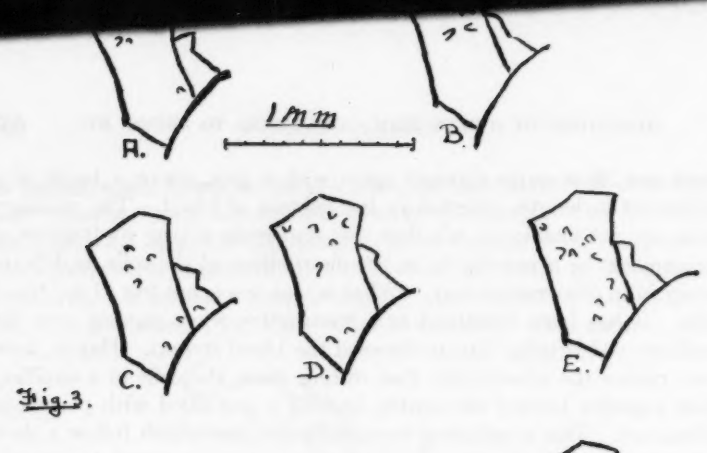

Fig. 3.

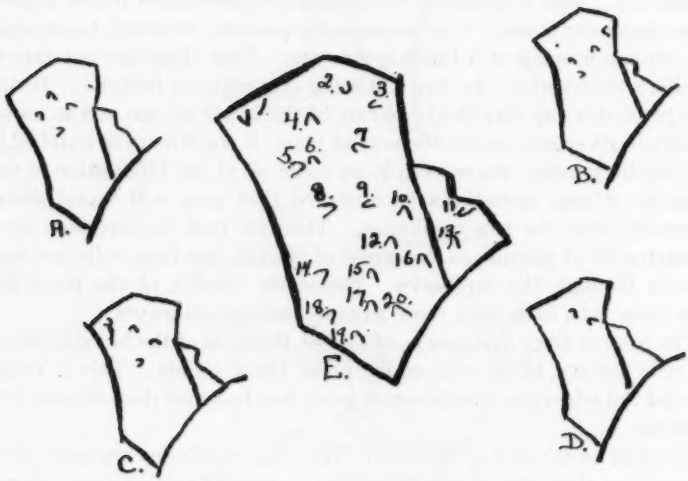

Fig. 4.

Fig. 3. The same area from the back of the hand observed at various times. The boundaries are made by the underlying vessels. In this area only the tips of the capillaries were visible.

A. December 12, 1921, 11:00 a. m. Capillaries *4*, *5*, *6* and *16* are open. (See numbered diagram, fig. 4.)

B. December 14, 1921, 11:15 a. m. Capillaries *7*, *8* and *9* are open.

C. December 15, 1921, 11:15 a. m. Capillaries *4*, *6*, *7*, *8*, *13*, *16* and *20* are open.

D. December 15, 1921, 12:15 a. m. Capillaries *1*, *2*, *3*, *4*, *7*, *8*, *19* and *20* are open.

E. December 15, 1921, 4:30 p. m. Capillaries *1*, *4*, *5*, *6*, *7*, *9*, *12*, *15*, *16* and *17* are open.

Capillaries *4*, *7* and *8* are open almost continually.

Fig. 4. The same field as shown in figure 3, drawn at intervals of 3 minutes.

A. 10:55 a.m. Capillaries *4*, *5*, *7* and *8* are open.

B. 10:58 a.m. Capillaries *3*, *6*, *7* and *8* are open.

C. 11:01 a.m. Capillaries *1*, *4*, *7* and *8* are open.

D. 11:04 a.m. Capillaries *4* and *7* are open.

E. 11:07 a.m. The field following light pressure.

All the capillaries are open. The diagram is drawn twice the size of the other figures and each capillary is given a number.

opening and closing of the capillaries goes on very rapidly, so that a drawing made every three minutes shows a different field each time (fig. 4). I have seen no observations of this shifting capillary field reported, though Lennartz (10) finds that mechanical stimulation opens capillaries previously closed, while Hintze (6) finds more capillaries open during light stasis than with a normal stream.

A further inspection of several microscopic fields clearly shows what may also be seen macroscopically, namely, that little areas a few millimeters in diameter, where only a few of the capillaries are open and the underlying vessels indistinct, alternate with fields where most of the capillaries are open and the underlying vessels prominent. The tissues in the former case are clear and white. Macroscopically these areas are seen as little white spots which go and come on the hand.

It should be emphasized that this picture of opening and closing capillaries is not that presented by those at the base of the nail. The majority of these are always open, though some of the smallest disappear temporarily, especially during cold.

Effect of temperature. Bruns and König (1) have described the appearance of the capillaries at the base of the nail, while the arm and hand are in water baths of various temperatures, ranging from 42° to 10°C. At the higher temperatures they report a rapid homogeneous stream in open capillaries. With lower temperatures the stream becomes slow and granular with wide gaps containing only plasma between the clumped erythrocytes. The capillaries are narrower. When the temperature is 10°, after 4 or 5 minutes, during which time the skin is pale, the capillaries contracted, they see a hyperemia recognizable macroscopically as well as microscopically. The capillaries are open and the blood stream moves forward.

Before I saw the work of Bruns and König I had examined microscopically the capillaries in a hand which was flushed with warmth, and also in a hand white with cold, and finally one blue with cold. At a room temperature of 25° the capillaries were all open and had an even rapid stream. At 20° there was a moderately fast stream in well-filled vessels at the end of the nail, while on the back of the hand there were the characteristic little white spots described above. If the temperature became much lower, however, a real contraction and relaxation of the capillary walls occurred. A contraction of the capillary, starting on the arterial limb and spreading gradually over the venous limb would narrow the column of blood. The blood stream, however, continued its flow, independent, in part at least, of this contraction in

the capillary wall. The lumen did not close entirely in this way, but only became narrower. The capillary sometimes widened again and the contraction was repeated. The arterioles at this time had disappeared, the veins were contracted and the skin pale. When the hands then became quite blue with cold, microscopically all the capillaries were open and filled with venous blood.

Bruns and König's experiments were then repeated. By putting a little oil on the skin first it is possible to cover it completely with water and still see the skin vessels microscopically. In a water bath of 34° the capillaries at the base of the nail had a rapid, even stream of blood flowing through them. At 20° the stream became granular, the capillaries contracted and there was soon a very slow stream in only a few capillaries and stasis in the remaining vessels. The arterioles had disappeared. In 10 minutes the hand was put into water at 10°. For 4 minutes there was little change in the contracted capillaries. Then the blood moved forward slowly in some of the vessels and for the next 6 minutes the capillaries gradually relaxed and filled with arterial blood, while the hand became red. The water was cooled again to 9° and after 10 minutes the blood stream, which had been moving more slowly, stopped. The hand became blue and all the capillaries, on the back of the hand as well, were open and filled with venous blood.

The changes in the skin vessels during a transition from a warm to a cold environment are therefore the following. When the surrounding atmosphere becomes more and more cold, the capillaries and arterioles, which in warmth are open and have an even, rapid stream become contracted. The flow of blood through them becomes very much reduced and the skin is white. Further cooling, however, paralyzes the capillaries so that they relax. For a time there is enough blood coming through the contracted arterioles to give a slow arterial stream in the capillaries; but, as the cold becomes more intense, the arterioles are so completely contracted that the flow of blood stops. The open capillaries fill with venous blood and the skin is cyanotic.

Effect of mechanical stimulation. Dermatographism. It is familiar to physiologists that, if the skin is stroked lightly with a blunt instrument, after a latent period of 15 or 20 seconds, depending on the temperature, the area in the path of the stimulus will blanch. This "white reaction" lasts several minutes. A harder stimulus results in a hyperemic line in the immediate path of the instrument. This "red reaction" is usually bordered on either side by narrow white lines. Müller (12) has shown that these reactions still occur when the segment of cord

supplying the area stimulated has been cut. They are not, therefore, of central nervous origin. Ebbecke (4) has anesthetized the ulnar nerve and found that the red and white reactions were still obtained in the area supplied by the anesthetized nerve. Since these reactions are not caused by central nervous stimuli, they may occur either because the stimulus affects the vessel walls directly, or because of local axone reflexes occuring peripherally, where the nerve has not been affected by the anesthesia.

Our preliminary observations on the effect of mechanical stimulation gave the folowing results. Light pressure in a microscopic field, with a fine pointed glass needle, opened all the capillaries of that field. This response was practically instantaneous. A field, which may have been quite pale with only a few capillaries open, immediately following light pressure will have all its capillaries open for the duration of a few seconds. The stimulus may be applied so locally that only one or two capillaries are opened by it. Such a very light pressure opens the underlying capillary loops for only 5 to 10 seconds, while a heavier pressure extending over a greater area (about 0.5 sq. mm.) may have an effect lasting 30 seconds. Sometimes the field thus stimulated, after initial flushing, becomes more pale than before the experiment. This was not constantly observed, however.

The effect of stroking the skin, as in the dermatographic reactions, was then tried on the back of the hand and the effect watched under the microscope. When a blunt point was passed lightly over a microscopic field, the initial reaction was similar to that described for light pressure with a point. The capillaries first opened up so that there were many more in the field than at the beginning of the experiment. Then, after about 15 seconds, they began to close and the white reaction came. As Parrisius (14) says, one of the most striking features is that the underlying plexus of vessels disappears also and the tissue background becomes more white and clear.

When the stimulus is so hard that the resulting reaction is a central red line with a white band on either side, the microscopic picture at first is the same as above. The capillaries open up both in the immediate path of the stimulus and to either side. In the course of 15 seconds, however, the central part, which has been, of course, more severely stimulated, becomes more flushed. The capillaries are fuller, the underlying vessels more prominent, and the tissue background is more red. But in the field to either side the capillaries and other vessels begin to close, and very soon the central red reaction and the

white reaction are well developed. It can sometimes be seen, without magnification, on the arm of a fair skinned person, that there is a general flush out of which the red and white reactions develop.

The additional information which these experiments give is therefore this, that the initial response to the mechanical stimulus is always an opening up of the capillaries, in the case of both the red and white reactions. In the case of the white reaction this is followed by a contraction, while in the red reaction the vessels become more open and full. This reaction includes the opening up of arterioles as well as capillaries, as can easily be seen on an arm cyanotic with cold. Here the red reaction has a brilliant arterial color. The white reaction cannot be obtained under such conditions of cold. The fact that the underlying vessels in the white reaction are contracted, while in the red reaction they are open and full, accounts for the major part of the color differences.

With the kind help of Doctor Hagedorn we carried out the following experiments to determine if local axone reflexes could be rsponsible for these two reactions. Doctor Hagedorn, on the arm of a normal subject, anesthetized an area about 5 cm. square, on all four sides, using a 2 per cent solution of novocaine. He thus obtained a central anesthetized field free from the disturbance of the experimental procedure. Mechanical stimulation had the following results. A blunt point drawn all the way through the anesthetized area and out onto the normal skin, gave the red and white reactions over the entire path, even directly above the sides of the square where the novocaine had been injected.

This evidence indicated that the red and white reactions were independent of local axone reflexes as well as central reflexes, as they occurred directly over the injected skin. To confirm this, an area of skin was anesthetized by running a fine needle very superficially and injecting 1 cc. of 2 per cent novocaine, and also, in two experiments, 5 per cent cocaine, so that there could be no doubt the most superficial sensory nerves were reached. When a light stroke was passed through such an area, the white reaction followed over the anesthetized area as well, though it took ½ to 1 minute longer for it to reach its maximum here.

These experiments over a carefully anesthetized area excluded all possibility that the sensory nerves were responsible for these two reactions. We cannot, however, be sure the sympathetic nerves were affected by this anesthesia. The possible causes for the red and white reactions are, therefore, reduced to two. Either they occur through

stimulation of the sympathetic nervous system or through direct mechanical stimulation of the vessel walls. There is no need to assume any other than a mechanical cause for the red reaction, which occurs in the area directly stimulated. In the case of the white reaction it may very well be argued either way. The fact that there was an initial flushing suggested the possibility that the capillaries were stimulated to contract by the increased supply of arterial blood. This possible explanation did not prove satisfactory, however, as the white reaction was also obtained when stasis had been first produced in the arm by applying a pressure cuff and raising the pressure to 200 mm. Hg. In spite of the fact that the vessels were thus cut off from their customary blood supply, the white reaction was obtained 10 minutes after raising the pressure.

Effect of certain drugs. From the above description of the reaction of the capillaries to mechanical stimulation and to temperature, it is evident that these factors must be taken into account when studying the effect of other stimuli. As the capillaries at the base of the finger nail, in contrast to those on the back of the hand, are practically unaffected by slight mechanical stimulation, this locality offered certain advantages. On the other hand, the capillaries at the base of the nail are less sensitive to chemical stimulation. Therefore all observations were made on both places.

The method of applying the drug is the following. A piece of glass tubing about 5 mm. in diameter is drawn out to a short, very fine capillary tip, a few hundredths of a millimeter wide. The tube is then partially filled from the top with a solution of the drug to be tested. This solution is blown through to the end of the capillary tube and the tube is then connected, by means of rubber tubing, with the top of one arm of a water manometer. By raising the leveling bulb, the water in the other arm also rises and the solution in the glass tube is put under a pressure corresponding to the difference in height between the two arms of the manometer. At a height of 20 cm. there is a pressure in the glass tube sufficient to force a very small drop of the solution through the fine tip, if the tip is lightly touched to the skin. It is possible, under the microscope, to slip such a fine capillary tube into the skin and bring the point near a single capillary loop, a venule or an arteriole. With such a tube containing a solution of the drug under 20 cm. H_2O pressure, it is therefore possible to bring a small quantity of the drug to be tested into direct contact with the vessel wall.

To control the mechanical effect of this procedure, a glass tube, filled in a similar way with saline, was repeatedly inserted at the base

of the nail alongside of various capillary loops and also by arterioles and venules. If the capillary tube were properly made, that is, if it had a short and very fine point it went through the skin without deforming it and could be brought to the side of a capillary without affecting it mechanically. To bring it alongside of an arteriole, the point had to be pressed deeper and the added mechanical manipulation sometimes hastened the stream for the duration of a few seconds. It was different, however, on the back of the hand. Here, as described above, any mechanical stimulation opened the underlying capillaries. A few, 12 or so, around the point where the needle was inserted, even when this contained only salt solution, remained open for several minutes, and were filled with blood. They gave even macroscopically a little flushed area. Therefore, in using the end of the finger, any increase in the size of the capillaries or in the rate of the stream, lasting more than a few seconds, could justly be attributed to the drug; while on the back of the hand an observed widening of the capillaries, unless considerable in extent, could not with certainty be attributed to any but the mechanical effect.

Effect of adrenalin. On the capillaries at the base of the nail adrenalin (Parke, Davis & Co.) in concentrations 1–1000 and 1–10,000, was effective in producing a contraction. The following is a protocol of a typical experiment.

A capillary glass needle containing a 1–1000 solution of adrenalin in 0.9 per cent saline is run into the skin so that its tip rests opposite the venous limb and at a point half-way down a straight capillary loop. It is removed in 10 seconds. In 15 seconds the part of the capillary opposite this point is contracted. The tip of the capillary is still visible and contains a few stagnant red blood cells. The circulation, as can be seen in the tip, starts again in 5 minutes; but the part of the capillary opposite the point of the needle is not back to its normal diameter for 45 minutes.

Adrenalin 1–1000 also produced contraction of the arterioles. The current was stopped at the longest for 7 minutes; this arteriole did not regain its former diameter, however, for 15 minutes.

The effect of adrenalin 1–10,000 at the base of the nail was similar to that of the above solution, but of shorter duration. The blood in a capillary so contracted would begin to flow in about 4 minutes and the loops returned to their former diameter in about 30 minutes; adrenalin 1–20,000 produced no effect. Higher dilutions up to 1–5,000,000 produced no certain effect. These very weak solutions were used in

order to see if it were possible, by this method to repeat the observations on man made by Dale and Richards (3) on cats. They obtained dilatation with such weak solutions of adrenalin.

The capillaries on the back of the hand were more sensitive to the effects of this drug. A solution of 1–1000 produced a small white area ½ to 1 cm. in diameter. A few of the capillaries immediately around the point of injection remained open for several minutes and gradually became blue. A solution as dilute as 1–100,000, after a latent period of 5 minutes, produced a white area 0.4 cm. in diameter. This reached its maximum intensity in 10 minutes and was still visible 15 minutes later. Solutions 1–1,000,000 and 1–5,000,000, could not be distinguished in their effect from a saline solution. The capillaries immediately around the point of the needle became dilated and remained so for some time.

In this area of the skin on the back of the hand it is not possible actually to see the contraction of the capillaries, as at the base of the finger nail. There is the possibility that the capillaries are open and contain only plasma. Krogh (9) has found, in the web of the frog's foot, that "the blood in a partially contracted arterial branch and the capillaries supplied by it may become anaemic, even to the complete disappearance of all corpuscles, by skimming off the plasma from the marginal zone of the main artery." The fact that the capillaries at the base of the nail were actually seen to contract makes this explanation very improbable, however. The evidence indicates a true capillary contraction on the back of the hand also.

Sollmann and Pilcher (18) have suggested that the blanching due to adrenalin may be caused by contraction of the arrectores pilorum muscles and compression of the vessels. This explanation is also unnecessary in view of the capillary contractions actually seen at the base of the nail.

Effect of histamine. Histamine 1–1000 tried at the base of the nail gave widening of the capillaries and hastening of the stream. This latter can only be explained as due to a widening of the arterioles also. The fact that those capillaries are always open and well filled made it impossible to obtain a very marked contrast between their original condition and that following a drug which produces dilatation. The effect was therefore confirmed by observations on several subjects.

On the back of the hand the needle with a 1–1000 solution of histamine produced, after a latent period of a few seconds, a sharp painful itching, which lasted 1 to 5 minutes (one subject 15 minutes). Around this point, for a radius of 1 to 2 cm. there was a bright arterial flush of a

very irregular shape. After about 1 minute, directly over the point of injection, a little white edematous bleb came, about 0.4 cm. in diameter. In the course of half an hour the bleb became smaller and red, finally fading into the surrounding flush. Both disappeared in about 1 to 1½ hours. Microscopically the capillaries, immediately around the point of the needle, filled and the stream became more rapid, as in the case of salt solution. In the course of a minute they became gradually fainter, the underlying vessels disappeared, and at the end of a minute only the tips of the capillaries could be seen, the edema and resulting increased thickness of the intervening tissue having obscured the field. In the surrounding flushed area all the capillaries were open and had a rapid stream. Histamine 1–5000 and 1–10,000 produced the same effect, but to a less pronounced and lasting degree.

Effect of Laportea. Needles from the poisonous plant, Laportea, were also examined. These little spines, about 3 to 4 mm. long, contain a fluid in their stem. If one of these was pushed through the skin there followed, with a few seconds' latent period, a very painful biting, itching sensation, which lasted for several hours and could even be noticed the following day or two. For a distance of 2 cm. or more around the point there was a bright irregular arterial flush, while directly over the site of injection a white bleb, similar to that observed with histamine though larger, formed in the course of 1 minute. The effect resembled that of histamine, but was much more intense and enduring. Fluid taken from the bleb through a fine capillary tube gave a strongly positive reaction for protein.

Effect of pituitrin. Krogh and Rehberg, in experiments not yet published, have found that, by perfusing the leg of a frog with very weak solutions of commercial pituitrin, diluted even to 1–100,000, they obtain a contraction in the capillaries of the web. It was therefore of interest to test solutions of this same pituitrin on the human capillaries.

At the end of the finger nail pituitrin (Parke, Davis & Co.) undiluted, gave a contraction of the capillaries. No effect on the arterioles could be seen. On the back of the hand in dilutions of 1–20, 1–50 and 1–100, after a latent period of 2 to 3 minutes, the skin over the point of injection began to blanch. The whitening did not reach its maximum intensity for about 10 minutes and was then only of diameter 0.4, 0.2 and 0.1 cm. respectively. The durations of the blanching were about 1 hour, 45 minutes and 30 minutes, respectively. Microscopically the field appeared similar to that following adrenalin, with these two exceptions. First, the latent period for pituitrin was longer, and second,

the open capillaries around the point, where the needle was inserted, remained filled with arterial blood. Hypophysin from Meister, Lucius & Brüning, and pituglandol from F. Hoffmann, La Roche & Co., gave the same results, but not so extensive or lasting.

On the back of the hand the three drugs described above are therefore alike in that, together with the plant Laportea, they produce a blanching. Histamine and Laportea also cause edema, which is sufficient to raise a bleb. Hence this is probably responsible for the blanching, because the pressure in the tissues is raised and the vessels are mechanically compressed. If the primary effect of histamine on the back of the hand were not dilatation, the results here would not agree with those obtained from the neighboring capillaries at the end of the finger.

The effect of stimulating these white areas mechanically and observing the result under the microscope was tried, in order to determine if they would all respond in a similar manner. If the white bleb of histamine or of Laportea was stimulated, by pressure with a blunt needle, the capillary loops became more prominent and were of a bright arterial color. This was only transitory and in 1 to 2 minutes only the tips of the vessels were again visible. Macroscopically the white bleb flushed pink. When the white spot produced by adrenalin was stimulated in a similar manner, a number of the capillaries appeared for a minute or more. These were of a blue color, however, and so were presumably filled with venous blood. Macroscopically there was nothing to be seen. The area blanched by pituitrin also responded to mechanical stimulation by the opening up of a few capillary loops. These, however, were of an arterial color. The effect here had also to be observed microscopically, as very little if anything was to be seen without magnification.

The blanching caused by adrenalin, histamine and pituitrin are evidently not of the same nature. Light stimulation following histamine produces a bright arterial flush. When adrenalin has been used only a few capillaries, filled with venous blood, are visible after stimulation, while a pituitrin spot responds by the appearance of a few capillaries, which are filled with arterial blood. In the case of histamine the capillaries are presumably all relaxed, but compressed by edema. The mechanical irritation is sufficient to open the arterioles and raise the pressure in the capillary bed to a point where the capillaries became filled in spite of the increased tissue pressure. As on the back of the hand, the dilatation due to a drug cannot be unequivocally distinguished from that due to mechanical stimulation, it is impossible to say with

certainty that histamine dilates the vessels here. Judging from the reaction at the base of the nail, it is most probable, however. A possible explanation of the edema is that the dilatation caused by this drug, or the drug itself directly, alters the permeability of the capillaries, so that plasma escapes. Dale and Laidlaw (2) have demonstrated transudation of plasma following histamine. The red flush surrounding the white bleb must be due to a nervous reflex produced by the pain.

Adrenalin and pituitrin are both similar in their action in that they cause a contraction of the capillaries. Only a very few become visible after mechanical stimulation. But in the case of adrenalin the arterioles also are contracted, so that those capillaries which open, fill with venous blood. The arterioles may also be contracted by pituitrin, but not so strongly as in the case of the former drug; for the stimulus employed is sufficient to open the arterioles and thus to supply the few open capillaries with arterial blood.

Effect of acetylcholine. Acetylcholine, 1–5000, at the base of the nail, if placed at the tip of a capillary loop, produced no effect. If the point of the needle was placed above an arteriole, however, the arteriole widened, the current became more rapid and the capillary also yielded slightly to this increased pressure and widened a little. In a dilution of 1–10,000 the effect was the same, though not so pronounced.

On the back of the hand acetylcholine could not be distinguished from salt solution.

Effect of amyl nitrite. Amyl nitrite, undiluted, applied in the same manner at the base of the nail, produced an increase in the rate of the current and a slight widening, which was more pronounced on the venous side of the capillary loop. The effect even with this undiluted solution was not great however. As in the case of acetycholine, on the back of the hand it could not be distinguished from salt solution.

Amyl nitrite when inhaled produced a very rapid stream in the capillaries, which were all open, but only slightly dilated. The effect must therefore be not only on the capillaries, but also on the small arteries.

Effect of urethane. A 20 per cent solution of urethane was tried at the base of the finger nail. There was a widening of both capillaries and arterioles and an increase in the rate of flow.

Discussion. No general conclusions as to the action of these drugs should be drawn from the results above. Not only different species of animals, but different parts of the same animal react differently to the same drug. Krogh (9) reports that adrenalin on the web of the

frog's foot causes contraction of the large arteries, and in the frog's muscle contraction of all the arteries while on the tongue (8) there is only dilatation. Histamine produces no effect in the web, when applied by a similar method. Moreover various concentrations of the same drug do not necessarily produce the same effect even in a given part of a given animal. As an example of this are the experiments of Dale and Richards (3), who found, in very weak dilutions, adrenalin produced dilatation of the vascular system in cats, while more concentrated solutions gave contraction. Until the nature of these reactions is better understood, it is therefore necessary to investigate each species individually.

The point which these reactions serve to illustrate, however, is the power of the capillaries independently to contract or relax. The contraction produced by adrenalin is so local that it may include only a portion of a capillary loop. On the other hand, the dilatation of arterioles due to acetylcholine produces practically a negligible dilatation in the capillaries. The difference in the action of pituitrin and adrenalin, one affecting capillaries, the other capillaries and arterioles, has already been described.

The active contraction and relaxation and the shifting number of open capillaries is very important in the regulation of blood distribution and the supply of the tissues, as has already been discussed by Krogh (7).

Of the experiments in the field of dermatographism, the ones which should be emphasized are first, those with very light pressure, where only a capillary or two open. This is a reaction of the capillaries themselves, which must be quite independent of any change in the arterioles. Then it was observed on a cold arm that, contrary to former belief, the direct red reaction included arterioles as well as capillaries. If the cause of the direct red and white reactions was not placed with absolute certainty outside the action of the sympathetic nervous system, it was at least made very probable that they are due to direct mechanical stimulation of the vessel walls.

This paper offers no experimental support to the theory that a peristaltic-like activity of the capillaries exists or may be regarded as a normal factor in forwarding the blood stream. But the capillaries can and do contract or dilate independently of arterial pressure and in response to local or general stimuli, thus constituting a factor of great importance in the regulation of the circulation.

SUMMARY

Using Lombard's method the smallest vessels in the human skin have been examined microscopically. By this method the rate of flow, the character of the stream and the state of contraction of the capillaries can be seen.

On the back of the hand normally only a part of the capillaries in a given field is open at one time; but all may easily be stimulated to open by light pressure.

Cold produces a contraction of arterioles with a slowing in the blood stream and contraction of the capillary wall. When the cold becomes extreme, the capillaries are paralyzed, open up and fill ultimately with venous blood.

Stroking an area of skin with a blunt point opens all the underlying vessels. This initial flush is followed by the usual dermatographic reactions. The red reaction on a hand blue with cold has a bright arterial color, showing that it involves arterioles as well as capillaries. Anesthesia experiments excluded the possibility that these reactions are due to sensory local axone reflexes.

The effect of the following drugs was tried, by using a fine capillary glass needle which could be run into the skin and brought to the side of a single capillary loop.

Adrenalin 1–1000 up to 1–100,000 produced contraction of both capillaries and arterioles.

Histamine 1–1000 up to 1–10,000, at the end of the nail, produced dilatation of the capillaries with hastening of the blood stream. On the back of the hand the effect was complicated by edema.

Pituitrin up to 1–100 produced contraction of the capillaries.

Acetylcholine, 1–5000 and 1–10,000 dilated both capillaries and arterioles.

Amyl nitrite, undiluted, dilated the capillaries slightly and increased the rapidity of the stream. When inhaled, all the capillaries were open with a very rapid flow.

Urethane, 25 per cent, dilated both arterioles and capillaries.

All the observations agree with the view that the diameter of the capillaries is not necessarily dependent on the pressure in the arterioles, but rather on their own tone and state of contraction. They normally contract or relax independently of the pressure behind them, but in response to local or general stimuli.

I wish to express my thanks to Professor Krogh and Mag. Rehberg, without whose help and advice the work would have been quite impossible.

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THE RESPONSES OF FUNDULUS TO WHITE, BLACK AND DARKNESS

G. H. PARKER AND A. J. LANCHNER

From the Zoölogical Laboratory, Harvard University

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It is well known that the common killifish, Fundulus heteroclitus (Linn.), will change in tint from a creamy white to a mottled steel-blue and back again according to circumstances. When Fundulus is kept in the laboratory in a white dish under bright illumination, it takes on a light creamy tone and remains so indefinitely. On etherization it quickly assumes the mottled steel-blue coloration. These two conditions represent the extremes of color change for this species and are easily excited in the laboratory.

To ascertain how much the illumination of the environment influences the tint of Fundulus, the following simple tests were tried. Three square wooden boxes were arranged so as to represent three different environments. The first box, which may be designated as the white box, was lined with dull white paper and one side was left open so that the interior could be illuminated by an incandescent electric lamp (Mazda, 100 watts). This lamp was placed in front of the open end of the box at about 60 cm. from its center. A second box, the black box, was like the first in all respects except that it was lined with dead-black paper instead of white. The third box, the dark box, was arranged so that it could be closed and was made absolutely light-proof. These three boxes provided, then, a brightly illuminated white environment, a brightly illuminated black environment, and an environment of complete darkness. The three boxes were set up in a large dark-room so that the surroundings had no special influence on their interiors.

In preparation for the tests a number of killifish were kept for several days in white dishes exposed to daylight. As a result of this treatment these fishes became light-colored and retained this tint persistently. Thus a constant condition was established from which each test could start. Three large battery jars were filled with water and a few of these uniformly light fishes were placed in each jar. One of these jars

was put in the white box, another in the black box, and the third in the dark box. They remained under these conditions for 24 hours.

It was expected that after the tests, the fishes in the illuminated white box would be found to be light-colored, those in the illuminated black box deeper in tint and those in the dark box near the extreme of mottled steel-blue. On examining them, it was found that those in the white box were light-colored, those in the black box dark in tint, both as had been anticipated, but those in the dark box were as light-colored as the fishes in the white box. These states were very evident when all three jars, after a test had been completed, were placed side by side on a white background in full daylight. The fishes from the black box were decidedly dark in tint and always easily distinguishable from the other two sets which, strangely enough, were identical in tint. After a few seconds, however, the fishes from the dark box, though they were on a light background, grew dark reaching a maximum in about half a minute. They then remained dark for about five minutes, after which they took on the light tint normal for their surroundings and remained indefinitely in this state. Thus the fishes from the white box and from the dark box, though similar in tint at the beginning, could be distinguished finally in that those from the dark box showed a temporary depth of tint. In the course of the past winter these tests were repeated many times and with remarkably uniform results. Fundulus in the dark is light-colored and on exposure to the light shows a temporary darkening.

Several modifications of the experiment described in the preceding paragraphs were tried. A number of fishes were prepared for testing by being placed in the black box till they were dark in tint after which some were put in each of the three environments for 24 hours. The results were absolutely the same as in the first experiment showing that dark fishes put in the dark box become light-colored and on subsequent exposure to light exhibit a temporary darkening. The element of time was also tried out by extending the tests to 5 days, but even after this period the results agreed exactly with those of the tests that extended over only one day.

An attempt was next made to ascertain what organs were concerned in these changes. From the conditions of the experiments it is possible that the alterations in the tint of the fishes might be due either to the direct action of light on the melanophores in the fish skin or to the indirect action of this agent on the melanophores through the eyes and nervous system. To test this question fishes were provided with collo-

dion blinders. Silk thread was stitched into the superficial skin immediately around the eyes and, after drying the head, collodion containing much lamp-black was dropped on the eye. This mixture caught in the adjacent threads and formed an opaque shell over the eye where it remained adhering for about a week. Fishes thus temporarily blinded remained light-colored as normal fishes on a light background do. They did not change from this state when put in the white box, the black box, or the dark box and on being taken out of the dark box and put in the light, they showed no temporary darkening. Evidently the type of response dealt with in these experiments is not a direct one in which the melanophores react immediately to light, but is an indirect one in which the eye is the receptor and the melanophore the effector.

An examination of the scales of the fish in the two extreme conditions seen in these experiments showed that in the creamy white state the melanophores were approximately spherical, though perhaps not as completely so as under certain other circumstances. In the dark condition the melanophores, as was to be expected, were expanded, in that their processes were filled with melanin granules though by no means so completely as in the steel-blue condition produced by ether. Thus the contracted and the expanded states of the melanophores of Fundulus in these experiments were determined by nervous activity in which the eye served as the receptor; the condition in this organ produced by a white environment or by darkness induced a contraction of the melanophores, that produced by an illuminated black field induced a partial expansion.

It would be premature to speculate on the relation of these responses to theories of vision. No serious student of retinal physiology regards any of these theories as satisfactory. Even the classical views of Young and of Helmholtz, of Hering, and of Ladd-Franklin are recognized as having elemental defects. But any one who can take consolation in the conclusion drawn from our observations that seeing black is more provocative of change than being in the dark is welcome to do so. In our opinion the change induced in Fundulus by the illuminated black field involves a question of contrast instead of one of simple vision and from this standpoint is rather a subject for further investigation than for present speculation.

PROTOPLASMIC EFFECTS OF PAPAVERINE, HISTAMINE AND OTHER DRUGS, IN RELATION TO THE THEORY OF SMOOTH MUSCLE CONTRACTION

HOYT S. HOPKINS

From the Department of Physiology, Johns Hopkins University Medical School

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While experimenting to test the toxic properties of histamine on protozoa, my attention was attracted by certain tonus effects produced by this drug, suggesting in a way its well-known constrictor action on smooth muscle. I decided to study this phenomenon further, eliminating all osmotic influences; and to compare the action of histamine with that of other smooth muscle stimulants like morphine on the one hand, and of such smooth muscle depressants as papaverine on the other. The results obtained point to the possible explanation of smooth muscle contraction in terms of definite protoplasmic changes, for the material afforded opportunity to compare the water-holding power, and hence the volume, of living organisms, as registered by the increase or decrease in size of their definitive vacuoles.

By far the most favorable material found for such a study was a strain of Spirostomum teres (fig. 1, *A*). Paramoecium, and sometimes other forms, were employed for comparison. Cultures of these organisms growing in a hay-infusion of distilled water were used, and also cultures of Paramoecium and Astasia in Ringer's solution diluted to 1/40. Solutions of the drugs, in lethal and sub-lethal concentrations, were made up in distilled water, and employed in neutral, acid and alkaline solutions within a narrow range of pH.

I. EXPERIMENTS WITH PAPAVERINE. The action of this drug will be considered first, as it illustrates most strikingly a series of morphological effects difficult of reproduction by the ordinary physical agencies.

a. Spirostomum. The most outstanding effect in Spirostomum produced by lethal doses of this drug is cytolysis. The animals disintegrate and disappear as if by magic, nothing remaining but a train of granules and the ovoid nucleus. Papaverine-hydrochloride (Hoff-

man-LaRoche Co.) was used; and since the solutions in distilled water were acidic, they were neutralized by adding just enough sodium-carbonate solution to give the desired reaction. The results obtained using neutral solutions are essentially like those with more acid solutions. With increasing alkalinity, however, the cytolytic effect becomes more and more pronounced. The more rapid cytolysis can be attributed to the action of hydroxyl ions on disintegrating protoplasm. For, as mentioned below, these ions have a constricting effect on living organisms, opposite to that of papaverine; but at the time of death, dissolution occurs more rapidly than in the absence of the drug. The cytolytic power of alkalis can be demonstrated independently of any drug, although certain drugs appear to alter the consistency of protoplasm, and thus greatly influence the speed of disintegration when the animals die.

Using a neutral solution of 1:100,000, one may carry on prolonged observations, examining the organisms in a hanging drop under high or low power of the microscope. Some animals die after 20 minutes, others live as long as 2 hours. They show at first some avoiding reactions, swimming backward, but soon resume their normal movements forward. The cilia are not affected, even up to the time of death. The sudden contractions of which these organisms are capable, owing to the presence of longitudinal myoid fibrils in their ectoplasm, are not influenced by the drug. Animals respond to mechanical stimuli (jarring of the preparation) by shortening to less than one-half their length. The physiological behavior of these myoid elements suggests that they are similar to the fibrils of skeletal muscle.

The most characteristic effect of papaverine on the living animal, however, is the gradual increase in the water content, as indicated by the much enlarged vacuole at the posterior end in Spirostomum (fig. 1, *B*). The collecting vacuole, or canal, extending the entire length of the body, becomes wider and hence more visible. Often one or more supernumerary vacuoles appear posteriorly, merging with the canal, thus adding to its content. Sometimes this vacuolation extends throughout the protoplasm. Specimens in papaverine retain their normal irritability, and become gradually more transparent and "relaxed." Any longitudinal folds or wrinkles present on the surface tend to disappear completely after 5 or 10 minutes. The contractile vacuole no longer pulsates, and increases steadily in size. During the stage of maximal distention, although they may seem normal otherwise, the animals become quite sensitive to mechanical disturbances. A single

contraction of the myonemes (induced by jarring the culture) then serves to bring about the final rupture,—and in fact nearly every specimen observed is thus "set off" by some mechanical shock.

Cytolysis usually begins at the anterior end, the pellicle once ruptured undergoing complete and rapid dissolution (3 to 10 seconds). The process takes place so quickly in alkaline solutions (pH 7.7) that the posterior portion of the body is forced backward through the water, leaving

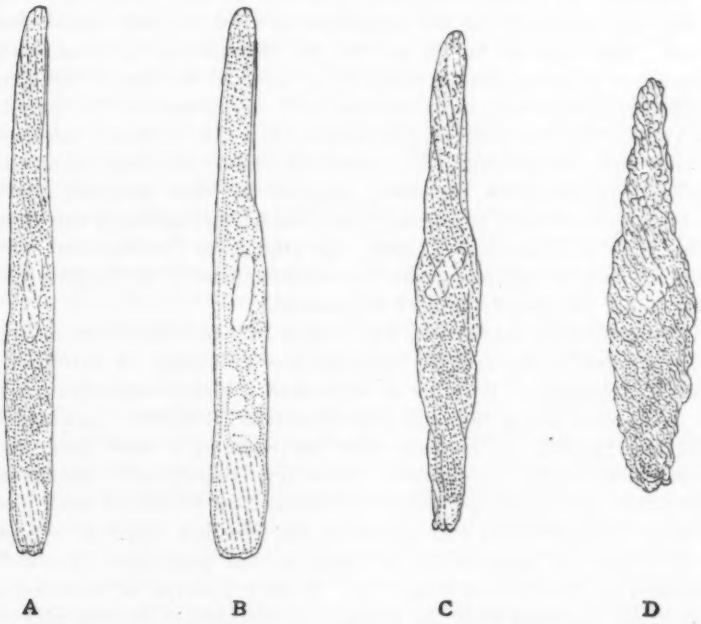

Fig. 1. Spirostomum, showing the diverse effects produced by drugs: *A* Normal individual; *B* an animal after treatment with papaverine-HCl, 1:100,000, showing vacuolation and distention of the body, but otherwise appearing healthy; *C* an animal 10 minutes after treatment with histamine-phosphate, 1:1000, showing constriction of the body; *D* dead animal, after treatment with histamine, in a shrunken and coagulated state.

a train of granules in its wake. It suggests most strikingly the appearance of a rocket in the air. In an acid medium (pH 6.6) the cytolysis is slower and more interrupted, but is usually complete.

The optimal conditions for distention in living animals are obtained in neutral solutions. The contractile vacuole, enlarging, pushes the protoplasm forward, so that the body is distended. Accessory vacuoles, representing an accumulated store of water, then form, each one merging with the contractile vacuole as its place is taken by smaller ones. In more acid solutions the swelling is not quite so marked, and the backward movement of vacuoles is retarded. This indicates a more coagulated condition of the protoplasm,—as does also its reduced tendency to cytolyze. In alkaline solutions (pH 7.8) another factor enters in, so that any tendency for the organisms to swell is partly counterbalanced. This appears to be due to the stimulating, or constrictor, influence of hydroxyl ions as such, for an aqueous medium of the same alkalinity induces marked constriction, with compression of the vacuole. Such effects might conceivably be osmotic, but this is quite improbable because they are produced by extremely dilute solutions of alkalis. Studies with histamine, moreover, support the view proposed above, for hydroxyl ions tend to augment the (constrictor) action of this drug, rather than to antagonize. Lastly, my studies on Paramoecium were made, in part, on animals grown in a saline culture (1/40 Ringer's solution), hence not greatly subject to exosmosis.

b. Paramoecium caudatum. The action of papaverine can also be demonstrated in this species, although less strikingly, as cytolysis is slow or incomplete. Paramoecia withstand a higher concentration of the drug, some living 18 hours in a solution of 1:100,000. In a neutral solution, 1:20,000, the animals swim backward for a short time, then resume their normal movements. Some few (thigmotactic specimens) may settle and remain quiescent, but retain their powers of rapid locomotion. This probably corresponds to the so-called "narcotic or anesthetic effect" of papaverine on Paramoecium putrinum (aurelia?), described by Macht and Fisher (1). It may perhaps be regarded as a condition resulting from the absence of stimulation, for the cilia are not paralyzed. Almost immediately after treatment there appear just beneath the ectoplasm minute vacuoles extending nearly the entire length of the body. After 10 minutes these have enlarged so as to approximate the two contractile vacuoles in size. These latter go on pulsating, but increase somewhat in size. Animals thus treated, while showing greater vacuolation and a tendency to swell, retain a healthy appearance and swim in a nearly normal manner (1 to 2 hours).

Summarizing briefly, papaverine may be said to exercise its characteristic action on Spirostomum by rendering the vacuole walls incapable

of contracting, thus allowing water to accumulate in the body. The retention of water in this organism is very likely associated with a more relaxed condition of the body, in that the normal pulsating movements of the vacuole are paralyzed. In Paramoecium the supernumerary vacuoles make their appearance early, while the contractile vacuoles are still pulsating, thus indicating, perhaps, a direct influence of the drug on the water-imbibing power of the protoplasm, before any paralytic action is manifest. This is suggested, also, in Spirostomum by the increased tendency for the protoplasm to undergo solution (cytolysis) when the animal dies. But whatever the explanation may be, the end results are the same: a greater retention of water by the organism, and distention of the body.

II. Experiments with benzyl alcohol. The few observations made by the use of this drug (saturated solution) indicate that it has a paralyzing effect on Spirostomum. There is observed, however, an initial excitation, characterized by rapid locomotion and rhythmic pulsations of the vacuole; after which the movements become much slower and the vacuole enlarges. The animals appear to be truly narcotized, even the myoid fibrils losing their power to contract long before the cilia are immobilized. The ectoplasm becomes increasingly more hyaline and loses its striated appearance, indicating a partial solution of the myonemes. Minute vacuoles fill the endoplasm, the contractile vacuole enlarges, so that the volume of the body is visibly increased. Animals at death undergo no marked cytolysis.

III. Experiments with saponin. This drug was employed in order to compare its cytolytic action on protozoa with that of papaverine. Prowazek (2) showed that saponin causes the pellicle in Paramoecium to become raised and the body to swell. It undoubtedly attacks the lipoids in the protoplasm, thereby causing dissolution, as in hemolysis.

I have confirmed Prowazek's observations. Paramoecia (caudatum and bursaria) cytolyze rapidly in a 1:1000 solution. A 1:10,000 solution gives surface changes, with the appearance on the body of raised blisters free from granular protoplasm. There is some swelling with much distortion, affecting mainly the posterior region of the body. This end becoming enlarged and more hyaline, shows an adhesive tendency, and finally cytolyzes. The posterior contractile vacuole enlarges moderately, but no new vacuoles are formed, as is the case with papaverine. Spirostomum is very little affected by a 1:10,000 solution. In a 1:1000 solution there is moderate distention of the

vacuole, followed by cytolytic changes starting at the posterior end. There is no bleb-formation.

Judging from its slight susceptibility and its freedom from surface effects, it would seem that Spirostomum is less rich in lipoids than is Paramoecium. But to papaverine we find Spirostomum to be more susceptible, so that it might be said that papaverine affects more directly the non-lipoid constituents of the protoplasm. It may be that it influences the water-holding power of the entire protoplasm, and that this in turn determines the state of tonus of the cell. Benzyl alcohol occupies an intermediate position. It is least active in cytolysis, but its effects are not so strictly localized as those of saponin.

IV. EXPERIMENTS WITH HISTAMINE. While the action of this drug is less specific, and therefore not so easily demonstrated, it is no less real. The toxicity of histamine for protozoa is slight in comparison with its known effects as a smooth muscle stimulant. "Ergamine-acid-phosphate" (Burroughs, Wellcome & Co.) was used in this work.

a. Spirostomum lives indefinitely in solutions weaker than 1:2000. Physiological effects are noted after dilution to 1:10,000. Specimens show a tendency to shrink, and the best index of this change is the constriction and distinct shortening of the contractile vacuole, often leading to its nearly complete obliteration (fig. 1, *C*). If the animals be stimulated (mechanically) to contract, even greater constriction may result. This is a superimposed effect, following the shortening of the animal by myoid contraction, for more water is thereby forced out of the vacuole and its reformation is checked. Accompanying this tendency to shrink, the protoplasm becomes more opaque and appears to be reduced in volume. The animals become less sensitive to mechanical stimuli so that, when caused to contract, they undergo incomplete (myoid) contraction and a tardy relaxation. This is accompanied by the appearance of folds or wrinkles on the body, which disappear but slowly after elongation. The ectosarc seems to be rendered very rigid (viscous), which may account for the persistence of wrinkles on its surface. Another effect is the decreased rate of locomotion, the cilia becoming visibly retarded,—so much so in a strong solution that they can be seen individually in motion. The slowing is most apparent about 5 minutes after treatment, and is probably associated with a hardening action of the drug upon the ectoplasm, for this would account for the shrinkage which takes place about this time. These various changes come on after about 2 or 3 minutes, and persist about 20 minutes. The animals regain their former state of

relaxation and swim more rapidly, the vacuole becoming partially distended. The effects of stronger solutions differ only in degree from those of more dilute solutions. They are so pronounced that they can hardly be confused with possible osmotic changes. A dilute sodium-chloride solution leads to lateral compression of the vacuole, but not to any shortening in the longitudinal axis. The protoplasm becomes somewhat hyaline and clear in hypertonic media, rather than more opaque, as in histamine.

Death occurs only in relatively strong solutions (1:1000 or stronger). A characteristic effect then presents itself. The protoplasm in the anterior portion of the body, in animals about to die, assumes a refractive and coagulated state. At death a sudden shrinking takes place, involving first the anterior third of the body, then the posterior end forward to the middle. By this final process of "constriction" the last trace of water is forced out of the vacuole, the corpse shrinks to about two-thirds its previous length, and presents the appearance of having undergone heat coagulation (fig. 1, *D*).

Spirostoma in alkaline solutions of histamine (1:500, pH 7.5) show a more marked constriction, with ridges prominent on the surface of the body. Animals dying in such a medium undergo shrinkage, but disintegration follows. The cytolysis here, as in the experiments with papaverine, seems to be secondary,—an effect of alkali; although in this case it does not set in until 1 or 2 minutes after death, and proceeds most haltingly. The influence of the drug is to render the protoplasm less susceptible to the cytolytic change, so that it takes place much less rapidly.

In a slightly acid solution, however, while the contraction phenomena are less striking in the living animals, the shrunken bodies, after death, are most persistent. These are found in a hard, opaque condition 24 hours after death, and never undergo a true cytolysis.

b. Paramoecium caudatum. Some difficulty was experienced with this species in demonstrating any action of histamine, except in the higher concentrations. With a solution of 1:500 constriction effects are noted. The vacuoles become smaller, but not reduced in number. In the posterior region of the body lateral compression occurs. Opposite sides become pushed in, one side more strongly than the other, leading finally to a curved (spoon-shaped) posterior end. These compression effects are more distinct in alkaline solutions. Paramoecia from a culture medium of 1/40 Ringer's solution, when treated with histamine, likewise show compression, so it is not likely that the changes

are the result of the osmotic extraction of water. Just preceding death the body is most constricted, and in some specimens is sharply bent at the posterior extremity and angular in cross section. No disintegration follows, but the vacuoles become somewhat distended, at the time of death, and the body more swollen and opaque, due to secondary changes in the protoplasm.

c. Astasia. This tiny metabolic flagellate, occurring in a Paramoecium culture of 1/40 Ringer's solution, was subjected to a 1:500 histamine solution. In control experimental cultures, to which water was added, the organisms swam in normal spiral courses, or crawled upon the substratum. "Peristaltic" movements of constriction, starting at the anterior end and moving backward, can be seen in many individuals in these control cultures. In the treated organisms violent contractions may be seen; and more characteristic of these cultures, "antiperistaltic" waves, progressing forward instead of backward. These movements are more violent than the usual peristalsis, and persist for a long time under the influence of the drug, one wave following another before the preceding one passes off. This type of constriction occurs but rarely in controls, and indicates, it seems, a reaction to some form of stimulation. Similar constriction waves (moving forward) have been described by Massart (3) in the flagellates Eutreptia and Euglena, when treated with dilute alkali (K_2CO_3). In cultures of Astasia treated with histamine the movements of the flagella are slowed, and only a few individuals relax and swim about freely.

All these observations, using histamine, indicate a stimulating action of the drug, so that slow constriction effects, with the accompanying loss of water are manifest. There is also displayed a coagulating action on the protoplasm, just previous to and at the time of death.

V. Experiments with morphine, codeine and apomorphine. The action of these drugs was investigated because they are known to have a stimulating influence on smooth muscle tissue. While their effects on living protozoa are not striking, the lethal effects are comparable to those of a constrictor substance. In solutions of morphine or codeine sulphate, of 1:500 or stronger, Spirostomum dies in a drawn-up condition (contracture), and does not cytolyze. This effect is characteristic of histamine rather than papaverine, which causes relaxation and cytolysis. There is, however, no coagulation in the living protoplasm. The animals show increased locomotion, and often swim backward rapidly in spirals. There may be partial reduction in the size of the contractile vacuole, lasting 10 or 15 minutes; but

it is not so pronounced as with histamine, and less easily distinguished from an osmotic effect.

Paramoecium responds by increasing its rate of locomotion, and backs away in a spiral course. There is some lateral compression of the body, with longitudinal folds appearing. Due to the inpushing of one side more strongly than the other, the body becomes bent.

Apomorphine (hydrochloride) produces similar effects, but is much more toxic. Protozoa are killed by a 1:1,000,000 solution. In higher concentrations its stimulating action is very evident. Spirostoma die in a contracted state.

The effects produced by these three drugs seem to be due primarily to their stimulating properties, for they are not such as can be attributed to specific changes (e.g., coagulation) in the protoplasm.

DISCUSSION

It may seem premature to discuss the relation of these observations to the interpretation of smooth muscle phenomena, since no generally accepted theory of contraction has been evolved. Certain points, however, should be emphasized in their relation to previous experimental work. As a preliminary in studying the mechanism of contraction, the volume changes undergone by muscle cells have received much attention. In terms of Meigs' theory of smooth muscle contraction (4), relaxation is associated with a taking up of fluid by muscle fibers from the lymph; contraction, with a loss in volume, and the expulsion of fluid from the cells. His microscopic studies (5) on fixed preparations of smooth muscle tissue, and on fresh sections, led to the direct observation that contraction is accompanied by shortening, with no increase in diameter of the fibers, thus confirming his earlier belief, arrived at indirectly through experimental means, that the fibers really lose volume during contraction. Striated muscle fibers, however, were found to undergo no change in volume.

The observations recorded in this paper go to show that substances which produce relaxation, or paralysis, of smooth muscle may lead to a profound increase in the water-content of living protozoa, and thus to an increase in their volume. This is, in particular, the case with papaverine, which is perhaps the most effective depressant of smooth muscle-activity yet known. Its action on protozoa may likewise be considered as a paralytic one. The power of constriction in the ectoplasm, by which the contractile vacuole is periodically emptied to the outside,

becomes paralyzed, so that water goes on accumulating inside the body and leads finally to its distention. Substances belonging to another class—the smooth muscle stimulants—bring about a visible loss of water, as is indicated by a reduction in size of a protozoan structure, the contractile vacuole. This effect is accompanied by constriction phenomena, —and with histamine is followed by a partial or complete coagulation. In general, the action of depressant drugs seems to be attended by a decrease in protoplasmic viscosity. Those which cause constriction may lead to increased viscosity; but their primary action is probably a form of "stimulation"—however that may be defined. Coagulation occurs secondarily for it appears to be irreversible.

As to the specific action of the drugs very little can be said. Each seems to be a law unto itself: it may exercise some particular chemical effect, the physical changes occurring secondarily. Macht correlates the depressor effects of various opium alkaloids with the presence of the benzyl grouping, thus pointing the way to a chemical interpretation of their effects. And, as we have seen, different benzyl-containing compounds (papaverine, benzyl alcohol) exert on living protozoa a somewhat different action, although in general the same. It is difficult, moreover, to harmonize the action of hydroxyl ions with that of drugs on the basis of their physical action alone. Judging by their cytolytic effects, alkalis seem to lower the viscosity of protoplasm. But, paradoxically, they produce constriction phenomena in intact organisms. The difference may be due to the lack of penetration of the OH ions, so that they produce first stimulation, by their superficial action; and then, in disintegrating cells, solution of the protoplasm.

It is not out of place in this connection to call attention to the nature of the contractile elements in the ciliated protozoa. Many of these organisms are known to possess, in addition to their cilia, highly differentiated myoid structures, similar to the fibrils of striated muscle, by which sudden contractions of the body are brought about. We may need to recognize, also, in rudimentary form, a property of tonus-regulation, such as we find especially developed in the cells of non-striated muscle. The protoplasmic functions underlying these tonus changes seem to be the same in protozoa as in muscle, as is indicated by the parallel effects of hydroxyl ions and of drugs.

SUMMARY

The effects of several drugs on living protozoa are described. Papaverine causes an increase in the water content of the living animals, as

shown by the increase in the size and number of their protoplasmic vacuoles. This leads to swelling of the body. Such animals, at death, cytolyze rapidly. Benzyl-alcohol gives rise to similar changes in the protoplasm; but there is less swelling of the body and little or no cytolysis. Saponin exercises a pronounced cytolytic effect on the cortical layer in certain protozoa, without influencing vacuolation, as does papaverine. Histamine produces constriction of the body in living protozoa, with reduction in size of the protoplasmic vacuoles. The disintegration of the body in animals dying in histamine is greatly retarded, and coagulation of protoplasm is often observed. Morphine, codeine and apomorphine exert stimulating and constrictor effects similar to those of histamine, without influencing the rate of disintegration.

The effects of papaverine and benzyl-alcohol, insofar as they lead to vacuolation and increased volume in protozoa, can be correlated with the depressor action of these drugs on smooth muscle cells; and those of histamine, and to a lesser extent, of morphine, codeine and apomorphine, with their stimulating effects on smooth muscle.

The apparent relation between the volume changes produced in protozoa by drugs having specific effects on smooth muscle, and the volume changes supposed to occur in smooth muscle during contraction and relaxation, are discussed.

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A COMPARATIVE STUDY OF THE DIFFERENT METHODS OF ARTIFICIAL RESPIRATION

R. BURTON-OPITZ

From the Department of Physiology, Columbia University, New York

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The methods of artificial respiration employed at the present time may be arranged in two groups: namely, those which purpose to expand the lungs in the normal way by altering the position of the walls of the chest by an external force, and those which produce a distention of these organs by driving air under a certain pressure into the pulmonary passages. In 1868, B. Howard of New York advocated the following procedure in threatened death from asphyxia by anesthetics and other causes: The subject is placed in the supine position. The operator then kneels over the legs of the patient; places the palms of his hands upon the lower regions of the chest, and presses inward by permitting the weight of his body to be supported for a few moments by his arms. During the subsequent period of rest the wall of the chest is allowed to recoil, thereby drawing air into the pulmonary channels. This procedure is repeated 8 to 10 times in a minute.

The method of Sylvester (1858) is very similar to this one. The subject is placed in the supine position with the head and shoulders resting upon a thin cushion somewhat above the level of the feet. The operator then assumes a position at the head of the patient, facing him. He grasps the arms just below the elbows and moves them laterally outward and upward. Having retained them in this position for 2 seconds, he again moves them into their former position and presses them against the sides of the chest during an equal period of time. Evidently, the raising of the arms is intended to expand the lungs and to cause air to flow into them in the normal manner.

Calliano advises to retain the arms in the Sylvester position so as to keep the thorax in this favorable state continuously. He then presses at intervals of 3 seconds with the flat hands upon the sides of the thorax and hypochondriac regions. It is also stated that the arms may be placed in a convenient position at the sides of the body.

Among the other methods which have been advocated from time to time, mention need only be made of the one described by Schäfer, because it differs from those just discussed in several important particulars. Schäfer advises to place the subject in the prone position, so that especial use can be made of the bellows-action of the thoracic wall. The operator kneels over the patient and applies pressure to the lowest ribs by adjusting his hands upon this region and permitting the weight of his body to rest momentarily upon his arms. On removing the pressure, the elasticity of the tissues so compressed causes them to resume their former shape and position. Since this interval, as well as the compression, is about 3 seconds in duration, this procedure may be repeated 12 times in the course of one minute. To distinguish this method from the others, Schäfer characterizes it as the "prone pressure method."

In accordance with the measurements of Vierordt it has been concluded that a man of average size breathes 5000 to 6000 cc. per minute. If the respiratory rate is 12 to the minute, the volume of the tidal air must amount to about 500 cc. Schafer (1) has tested the effectiveness of his method by means of a Hutchinson spirometer. The results show that an adequate amount of air can readily be moved by a rate of compression of the chest very close to the rate of the normal respiratory movements. Contrariwise, Schafer could not obtain a sufficient interchange of air with the aid of any one of the other procedures.

Spirometric tests upon animals. I have recently devised a spirometer which registers the movements of the tidal air as a continuous line upon the smoked paper of the kymograph, showing every alteration in its volume that may have developed at any particular moment (2). Since this instrument is equipped with a double valve which may be interposed at any moment to prevent either the inspired or the expired air from passing through its central cylinder, separate records may also be made of the successive inhalations and exhalations. The extreme sensitiveness of this apparatus has prompted me to employ it in this series of tests which purpose to compare the volume of the normal tidal air with that forced into the lungs by different artificial procedures. In elaboration of this subject-matter I have also made a large number of similar tests upon cats and dogs, before as well as after death.

It must be admitted that it is practically impossible to retain a normal cat in a condition of comparative rest for a sufficiently long period of time to ascertain its respiratory capacity and to practice artificial respiration upon it. The procedure finally followed consisted in con-

necting the trachea of the etherized animal with the spirometer, and in determining the volume of the tidal air during normal as well as artificial respiration. The first factor was obtained during the lightest possible state of narcosis and the second, during deep narcosis. The artificial procedure was repeated after the animal had been killed by an overdose of the anesthetic.

While only slightly etherized, these cats yielded an average tidal volume of 69 cc. or 2070 cc. in a minute. When the anesthetic was pushed to produce an almost complete cessation of the respiratory movements, the volume of the tidal air decreased gradually until it assumed the value of 30 cc. shortly before the respiratory standstill.

The question then arose whether it is possible to satisfy the normal respiratory requirements of this animal by artificial respiration. A very simple procedure consists in placing the hands against the lateral aspects of the thorax in the region of the diaphragm and in exerting moderate pressure upon these parts. On releasing the pressure, air is drawn into the pulmonary passages in consequence of the recoil. The compression is repeated 25 to 30 times in a minute.

It became evident immediately that even a very moderate compression of this region of the thorax yields a normal tidal volume, provided the hands are placed in part against the hypochondriac regions. The values of the tidal air obtained at this time varied between 60 and 85 cc. Especially in young animals, it suffices to compress the chest from side to side between the tips of the fingers of the two hands. Contrariwise, the compression of the thorax between the thumb and index and middle fingers of one hand has proved wholly inadequate.

A more efficient procedure is to apply the basal portions of the thumbs to the epigastric area, so that the hypochrondriac regions and costal arches come to lie in the palmar depressions of the hands and the fingers extend well into the axillary spaces. If a gentle pressure is now exerted by both hands, the sides of the thorax will be flattened, causing the costal cartilages to bend somewhat and the sternum to move slightly forward. The carpal margins of the hands meanwhile oppose the downward displacement of the liver and stomach.

The amount of air which may be moved in this way by compressions of very moderate intensity greatly exceeds that ordinarily shifted by the normal respiratory movements. Accordingly, if this procedure is repeated about 20 times in a minute, the cat must receive a larger minute-volume of air than that obtained normally during light ether narcosis when the number of the respirations was 30 to the minute.

In fact, the normal tidal volume may easily be doubled by this means without exerting an excessive pressure. The succeeding table 1 will illustrate this point more fully.

The average weight of the cats employed for these tests was 3.4 kgm. Their average tidal volume in light ether narcosis amounted to 69 cc. Accordingly, their average minute-volume may be calculated at 2070 cc. or at 600 cc. per kilo of body weight.

TABLE 1

EXPERIMENT NUMBER	TOTAL QUANTITY OF AIR MOVED	NUMBER OF COMPRESSIONS	QUANTITY DURING EACH COMPRESSION	NORMAL TIDAL VOLUME	EXCESS
	cc.		cc.	cc.	cc.
1	1200	12	100	72	28
2	600	5	120	70	50
3	1800	20	90	65	25
4	600	4	150	72	78
Average				69	

In figure 1 a curve representing the quantities of air moved by the successive compressions, *A*, is registered in relation with a curve indicating the movements of the normal tidal air, *N*. A distance of 3 mm. in the excursions of the writing lever indicates that 70 cc. of air have traversed the instrument. It will be seen that the normal tidal volume can readily be doubled by the artificial procedure.

The results obtained by this method upon dead cats was not satisfactory. In order to attain a normal tidal flow in these animals, it became necessary to compress the thorax more completely and to increase the frequency of the compressions considerably. It appears that the thorough relaxation of the tissues leads to a considerable loss of pressure chiefly in the direction of the abdomen, which, however, may be partially prevented by bandaging its lower part. In general, it may be said that while it is a relatively simple matter to establish a normal tidal volume in narcotized cats, artificial procedures applied after death do not yield more than one-third the required amount of air. Consequently, the compressions must be greatly increased in frequency otherwise a normal minute-volume cannot be attained.

The conditions noted in dogs weighing between 6 and 8 kilos are practically the same as those just described. In the larger animals more strenuous efforts must be made, although it is still a simple matter to establish a normal tidal volume with each compression. The method

most certain to produce satisfactory results, without causing injury to the parts manipulated, consists in placing the flat hands upon the ventrolateral regions of the lower thorax and upper abdomen and exerting a moderate pressure in the direction of inward and upward. The carpal margins of the hands should be employed to push the liver and stomach upward, while the remaining portions of the hands are used to press the lower ribs inward. Owing to the relative inflexibility of the upper ribs, it becomes necessary to apply the hands at a rather low level,

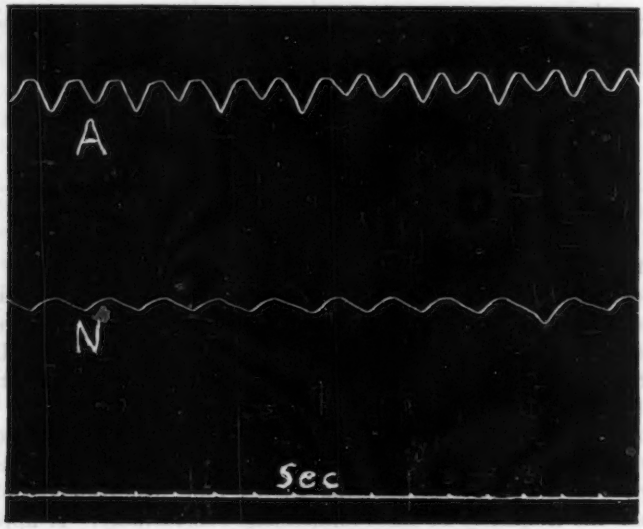

Fig. 1. The normal volume of the tidal air, *N*, in relation with the amount of air moved by artificial respiration, *A*. Time in seconds; 3 mm. of distance equal 70 cc.; not reduced.

thereby converting the area of each costal arch and adjoining hypochondriac and epigastric regions into the movable side of a pair of bellows. Under ordinary circumstances, an attendant should open the mouth of the animal and withdraw the tongue rhythmically, so as to enlarge the faucial orifice and to stimulate the respiratory mechanism, if possible, in this particular reflex manner. Obviously, since the trachea of each animal was connected directly with the spirometer, this manipulation was not resorted to during these tests.

A more strenuous but also more efficient method is the following: The dog is raised by the hind legs and is held free from the table, while another attendant opens its mouth and withdraws the tongue rhythmically. The operator then adjusts the animal in such a way that its back comes to lie against his abdomen while his hands encircle its hypochondriac regions and costal arches. A forceful pressure is then exerted in a direction inward and toward the animal's head.

The effectiveness of the method mentioned first may readily be proven by comparing the normal volume of the tidal air of a dog of average size with the volume of air moved by this artificial procedure. A number of tests made upon dogs in light ether narcosis in the supine position, have yielded the following average results:

TABLE 2

AVERAGE WEIGHT	NORMAL RESPIRATION		ARTIFICIAL RESPIRATION		EXCESS
	Minute volume	Tidal volume	Total volume	Per compression	
kgm.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
20	6400	214	7400	390	176

The three dogs used for these tests possess an average weight of 20 kgm. and an average volume of the tidal air of 214 cc. or 6400 cc. per minute. Hence, 320 cc. are apportioned to each kilo of substance per minute. It will be seen that the aforesaid method of artificial respiration yields a tidal volume considerably in excess of normal requirements, so that the frequency of compression may indeed be somewhat diminished without fear of establishing too slight a ventilation of the lungs.

This relationship is clearly apparent in figure 2, which embraces a record of the normal tidal air, N, and a record, AR, of the quantity of air moved by very moderate compressions. A movement of the writing lever of 10 mm. indicates the passage of 214 cc. of air. Even a very casual glance at this figure will show that the tidal flow during the compressions is about twice as large as the normal.

The effectiveness of the "supine pressure method," when applied to dogs, may also be illustrated with the aid of figure 3. The first curve, marked *normal*, indicates that 3600 cc. of expiratory air have traversed the spirometer, A to A. The inspiratory periods are recorded as straight lines. The movement of this quantity of air has consumed about 14 seconds or 22 expirations. Accordingly, the average expiratory volume may be calculated at 164 cc. The second curve, marked *art. resp.*,

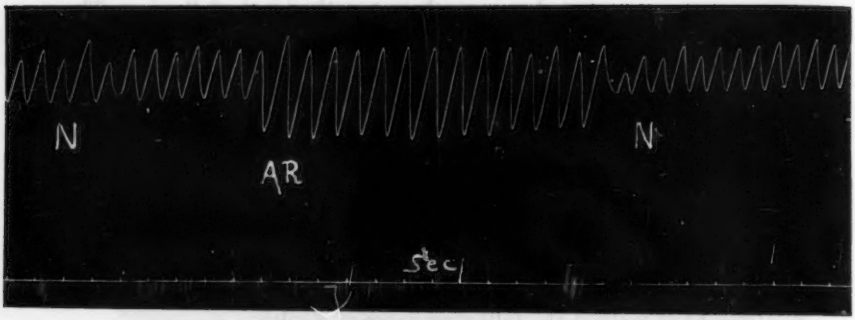

Fig. 2. The normal tidal flow, *N*, in relation with the quantity of air moved by artificial respiration, *AR*, in dog. Time in seconds; 10 mm. of distance equal 214 cc.; reduced two-sevenths.

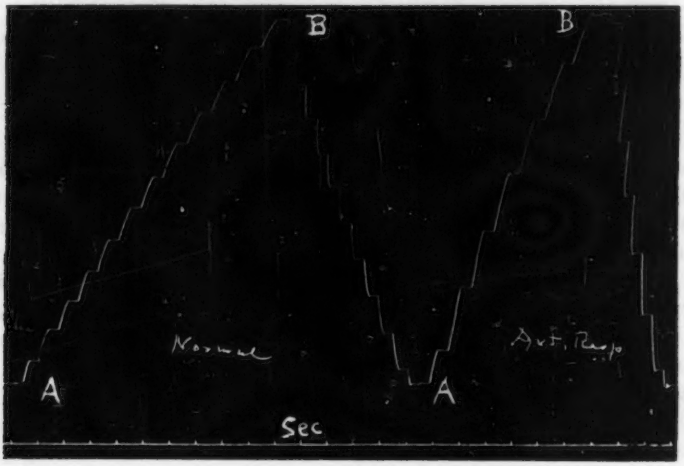

Fig. 3. Record of successive expirations in dog; normal and artificial respiration. Time in seconds. Distance *A* to *B* indicates 1800 cc. of air; reduced one-third.

also represents the movement of 3600 cc. of air through the spirometer. The thirteen compressions required to propel this quantity of air have consumed only 8.5 seconds. In the latter instance, therefore, each compression has yielded about 280 cc., as against the normal tidal volume of 164 cc.

Spirometric tests upon young men. The young men who were selected for these experiments purposing to ascertain the effectiveness of the different procedures of artificial respiration, have given an average height of 70 cm. and an average weight of 72 kilos. The subject was first placed in the supine position with the head slightly elevated and the arms adjusted at the sides of the body. After a certain period of rest, he was permitted to breathe through the spirometer for 2 minutes, so as to obtain a fair value of the volume of his tidal air. Shortly thereafter he was again connected with the spirometer. He then relaxed fully in partial inspiration, while the operator completed two or three artificial respirations. By this arrangement it was possible to obtain consecutive measurements of the amount of air moved normally and that moved by the compressions. Moreover, it seemed best to limit the number of artificial efforts so as to retain the subject in a proper state of aeration. Secondly, since this spirometer may also be adjusted in a way to register solely the inspiratory or the expiratory volumes of air, it was possible to ascertain the number of normal respirations required to move a definite quantity of air in a given period of time. This could then be compared with that representing the number of compressions necessary to propel an equally large amount of air during the same length of time.

Stress has been placed by Sylvester and Calliano upon the fact that the upward movement of the arms tends to expand the chest and to cause air to flow into the pulmonary passages. The present spirometric measurements prove that the amount of air which may be moved by this means, is so small that it cannot possibly serve a practical purpose.

It will be remembered that Sylvester recommends to bring the arms eventually in contact with the walls of the chest, and to press the latter inward. The succeeding discussion will show that this procedure fails absolutely in producing a normal interchange of air if repeated at the rate of the normal respiratory movements. Consequently, inasmuch as very little can be gained by the raising of the arms and inasmuch as the lateral compressions of the thorax cannot establish an adequate minute-volume unless repeated with undue force and frequency, the effectiveness of the Sylvester method may justly be questioned. It will also be remembered that Calliano advises to leave the arms in the extended position, and to confine one's efforts to the lateral compression of the chest. This modification causes his method to become practically identical with that of Howard.

The present spirometric measurements prove that the latter method is the only one which need be seriously considered in this connection. It will be noted that the patient is placed in the supine position with his arms in an easy position at a short distance from the sides of his body. The operator then kneels over the legs of the patient and presses with the palms of this hands vertically upon the lower region of the chest. The results of this procedure may be studied with the aid of table 3.

TABLE 3

EXPERIMENT NUMBER	AVERAGE VOLUME OF TIDAL AIR PER MINUTE (REST)	AVERAGE VOLUME OF TIDAL AIR PER RESPIRATORY CYCLE	AVERAGE VOLUME OF AIR FOR EACH COMPRESSION	DIFFERENCE
	cc.	cc.	cc.	cc.
1	6360	530	210	−320
2	5760	480	260	−220
3	5100	510	280	−230
4	4900	485	310	−175

It will be seen, therefore, that the method of Howard, when practised with proper care, yields only about one-half of the normal tidal flow. Accordingly, in order to be able to equal the normal volume of the respiratory air per unit of time, the frequency of compression must be increased to something like twenty-five to the minute. These measurements also show that the efficacy of this method may be progressively diminished by applying the hands at levels higher than that of the xiphoid cartilage.

In order to produce the most decisive effects, the hands should be placed upon the hypochondriac and epigastric regions, so that the tips of the thumbs come to lie below the xiphoid cartilage, while the fingers are extended laterally across the lower ribs. The carpal margins of the hands may then be used to exert pressure upon the organs occupying the upper extent of the abdominal cavity. Further, the compression should not be made in a direct line inward but inward and upward, because the principal path of attack is through the diaphragm. Lastly, the legs should be slightly flexed upon the trunk, so as to facilitate this particular manipulation. If these modifications are made, it is possible to equal about two-thirds of the volume of the normal tidal air. Accordingly, the rate of compression must still be greater than the normal, namely, about twenty to the minute.

In order to illustrate this statement more fully, I have inserted in this place figure 4 which compares the normal number of respirations required to move 1800 cc. of air with the number of compressions required to propel an equal amount. The letters *A* and *B* indicate that 1800 cc. of expiratory air have traversed the spirometer. The inspiratory periods are here represented as straight lines, because the inspired air was prevented by a valve from passing through this instrument. Curve *N* shows that three normal respiratory movements sufficed to move this amount of air; hence, the volume of the normal expiratory air amounted in this instance to about 600 cc. Curve *C* proves that five compressions were required to move 1800 cc. in an equal period of time. Accordingly, only 360 cc. of air were propelled during each compression. Supposing then that the normal rate of respiration is 12 in a minute when at rest, 20 compressions should be made to yield an equally large tidal volume.

The results obtained when the subject was made to assume the prone position were much more favorable than those just described. It will be remembered that Schafer recommends to place the subject flat upon his ventral surface, with his legs somewhat apart, and his arms in a slightly flexed position at the sides of his body. The operator then kneels over the legs of the patient and applies his hands to the latero-posterior regions of the lower ribs. He then permits the weight of his trunk to rest at intervals upon his arms.

The procedure followed to test the efficacy of this method was the same as that described previously. The patient having been connected with the spirometer, was permitted to breathe through this instrument for a brief period of time, in order to ascertain the volume of his tidal air. Under similar circumstances, a few compressions were then made at long intervals, so as to determine the amount of air which can be moved in this manner. As in the preceding tests, it was also noted how many normal respiratory movements and how many compressions are required to move a certain quantity of air in a given period of time. The results of these experiments are compiled in table 4.

It is evident that this procedure yields an ample quantity of air even if the rate of compression is not increased above normal, and furthermore, the force which must be applied each time to attain this end need not be considerable. Accordingly, these experiments fully confirm the statements of Schäfer which claim that the "prone pressure method" is the most effective. Whether it can be employed at all

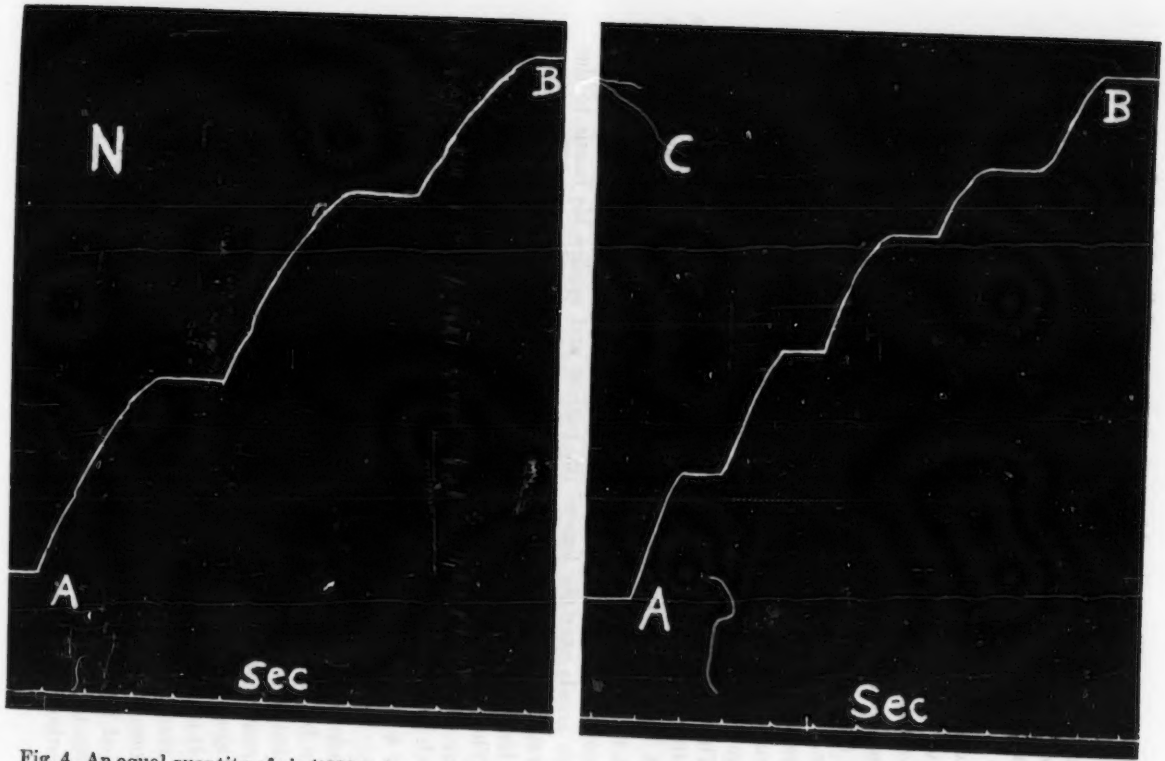

Fig. 4. An equal quantity of air (1800 cc.) moved *N* normally and *C* by artificial respiration. Time about 10 seconds each. Reduced one-third.

times depends, of course, upon circumstances. Thus, one would not use it upon persons whose abdominal cavity has been widely opened.

In order to increase the efficacy of this method and to avoid possible injury to the parts compressed, a wedge-shaped cushion, about 8 cm. in thickness, should be placed transversely across the abdomen so that

TABLE 4

EXPERIMENT NUMBER	AVERAGE VOLUME OF AIR PER MINUTE	AVERAGE VOLUME OF TIDAL AIR	AVERAGE VOLUME OF AIR FOR EACH COMPRESSION	DIFFERENCE
	cc.	cc.	cc.	cc.
1	6180	515	545	+30
2	6360	530	710	+180
3	5760	480	600	+120
4	5100	510	580	+70
5	4900	485	640	+155

the umbilical region of the subject rests upon its rounded ridge. The head should be permitted to project across the edge of the table and should be placed in a rest or held by an attendant in a somewhat dependent position. The raising of the umbilical region greatly diminishes the downward trend of the pressure so that the best possible bellows-effects may be obtained. Under these circumstances, it suffices to exert only a very moderate degree of pressure, provided the hands are placed flat against the lower ribs and the pressure is directed inward and upward. It is then an easy matter to exceed the normal volume of the tidal air by as much as 100 to 150 cc.

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THE EFFECT OF A HIGH PROTEIN DIET ON THE BLOOD CATALASE

W. E. BURGE AND J. M. LEICHSENRING

From the Physiological Laboratory, University of Illinois

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Lavoisier showed that the ingestion of food increased oxidation in the body. Rubner (1) found that protein was more effective in this respect than fat or carbohydrate, and termed this property the specific dynamic action of protein. Eighteen or twenty hours after a meal the food no longer exerts any stimulating effect on the oxidative processes and the animal is said to be on basal metabolism, that is, he is living on the material stored in his tissues. Rubner (2) found that when large quantities of meat were ingested for several days in succession the usual specific dynamic action occurred after each meal and also a rise in basal metabolism to a higher level. The object of the present investigation was to determine if the ingestion of a large meal of meat would increase the blood catalase corresponding with the increase produced in metabolism, and if the ingestion of large quantities of meat for several successive days would produce an increase in catalase corresponding with the rise in basal metabolism.

Dogs were used in the experiments. These animals were kept in cages of ordinary size and given exercise each day by running in a tread wheel for five minutes. The catalase determinations were made by adding 1 cc. of the jugular blood to 25 cc. of neutral hydrogen peroxide and the amount of oxygen liberated in ten minutes was taken as a measure of the catalase of the blood. Determinations were made of the catalase of the blood of the dogs on basal metabolism, while they were being kept on a mixed diet, as well as on a diet consisting solely of a large quantity of meat. The mixed diet was made up of fresh scraps of meat, bread, potatoes, pie, vegetables, etc., obtained from a large restaurant. The meat fed was round steak freed from adhering fat.

Detailed results from the determinations of the blood of one dog are shown in figure 1. This was a very vigorous dog, as were also the other

dogs used. He ran in the tread wheel very willingly for his exercise and partook of his food greedily. It may be seen that at 9 a.m. April 1, 1 cc. of the blood of the dog, which was then on basal metabolism and had been kept on a mixed diet, liberated 70 cc. of oxygen from neutral hydrogen peroxide in ten minutes. After this determination the dog was given five minutes' exercise and a little later fed as much of the mixed diet as he could eat. A similar determination was made April 2, at 9 a.m. and it may be seen that the blood liberated 69 cc. of oxygen. Similarly determinations were made on April 3, 4 and 5. From this it may be seen that the amount of oxygen liberated by 1 cc. of the blood of the dog while on basal metabolism and on a mixed diet was approximately 69 cc.

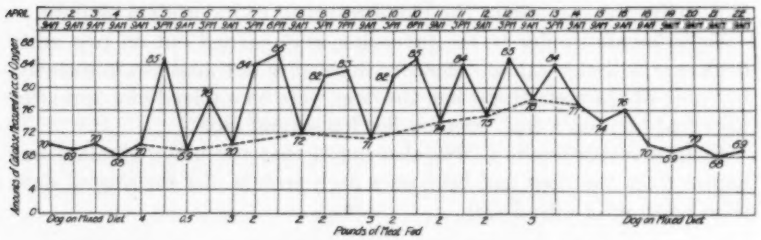

Fig. 1. Curve showing the catalase content of the blood of a dog on basal metabolism while being kept on a mixed diet and while being fed large amounts of meat for several days. The increase in the blood catalase after each meal of meat is also shown.

After making the catalase determination April 5, at 9 a.m., the dog was fed four pounds of round steak instead of the usual mixed diet and at 3 p.m. another catalase determination was made. It may be seen that 1 cc. of the blood liberated 70 cc. of oxygen at 9 a.m. and 85 cc. at 3 p.m. After making this last determination the dog was given the customary five minutes' exercise in the tread wheel and then placed in his cage until 9 a.m. the next morning, April 6. At this time the blood liberated 69 cc. of oxygen. Half a pound of meat was given the dog after this determination and at 3 p.m. 1 cc. of blood liberated 78 cc. of oxygen. The dog was again given five minutes of exercise and placed in his cage until April 7, at 9 a.m., when another determination was made and the blood liberated 70 cc. of oxygen. The dog was then given three pounds of meat and at 3 p.m. the blood liberated 84 cc. of oxygen from neutral hydrogen peroxide. Immediately after taking the sample of blood at

3 p.m. two pounds of meat were given and at 6 p.m. 1 cc. of blood liberated 86 cc. of oxygen. After this the dog was given exercise for five minutes and again placed in his cage where he remained until 9 a.m. April 8. In a similar manner determinations were made and meat fed on April 10, 11, 12 and 13. On April 14 at 9 a.m. the dog was again put on a mixed diet and it may be seen that the catalase content of the blood had returned to its original amount by April 18.

By examining the curve in figure 1 it may be seen that the blood of the dog liberated approximately 69 cc. of oxygen, while on basal metabolism and kept on a mixed diet, and that after the dog had been fed large quantities of meat from April 5 to April 13 the blood catalase rose so that 1 cc. of blood liberated 78 cc. of oxygen while on basal metabolism. From April 14 to April 22 when the dog was put on mixed diet again, the catalase of the blood returned to its original amount.

Experiments similar to the preceding were carried out on two other dogs except that catalase determinations were not made so frequently, but only at the beginning when the dogs were on a mixed diet and after they had been on a high protein diet for several days.

In these experiments it was also found that the blood catalase was higher when the dogs had been on a high protein diet for several days than when on a mixed diet.

SUMMARY

The ingestion of a large meal of meat produces an increase in catalase parallel with the increase produced in metabolism.

The ingestion of large quantities of meat for several successive days increases catalase corresponding with the rise produced in basal metabolism.

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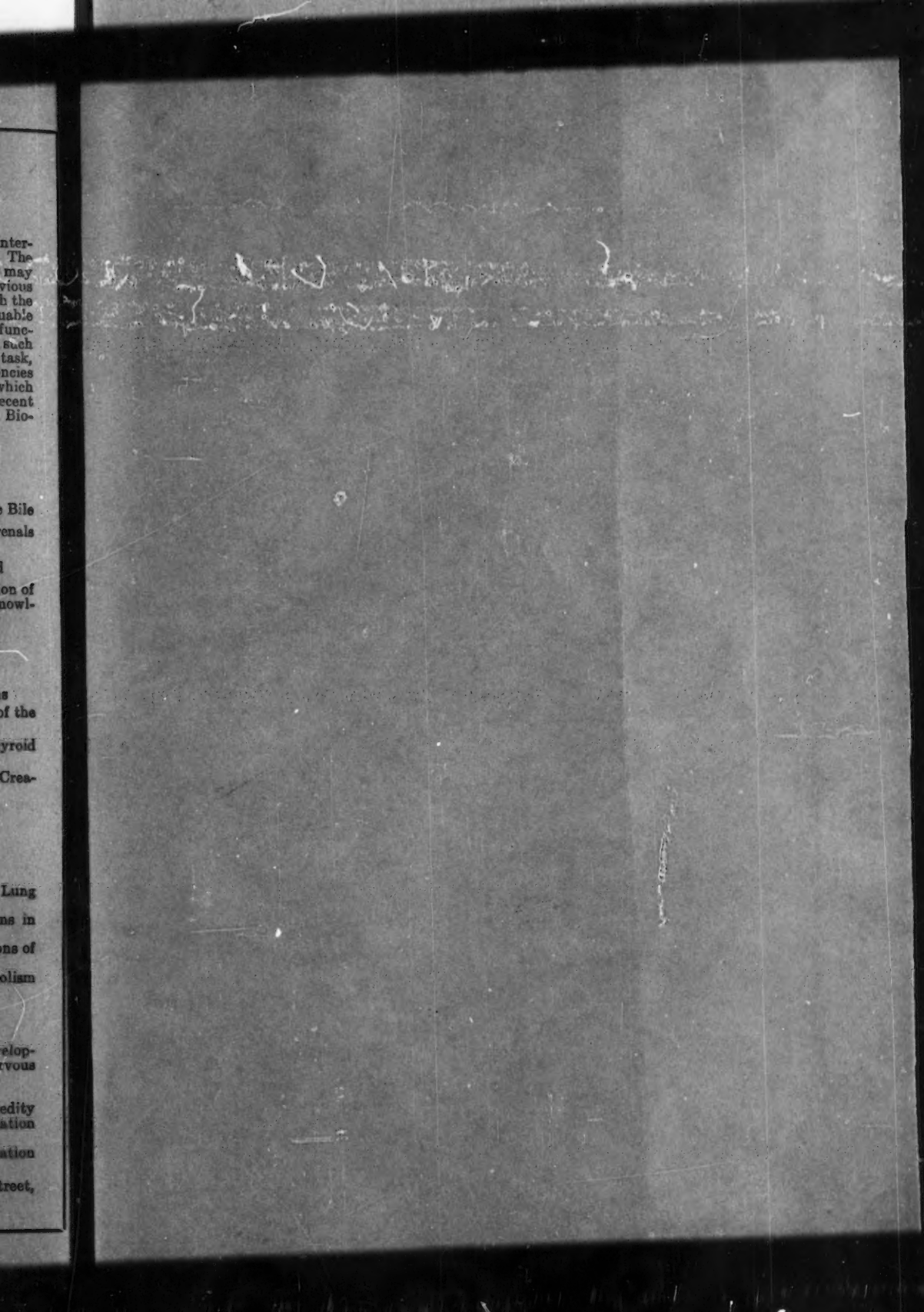
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